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THE PENETRATION OF 3:5-DINITRO-*o*-CRESOL INTO LEAVES

BY G. E. FOGG*
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(With 1 Text-figure)

A study of the penetration of 3:5-dinitro-*o*-cresol from spray solutions or suspensions into leaves of *Sinapis arvensis* L. shows that entry through the stomata is unimportant. Lethal amounts may enter from aqueous solution by simple diffusion of the undissociated phenol through the epidermis, which behaves as a homogeneous lipide membrane. The effect of the structure of the epidermal surface on the rate of diffusion is discussed. Lethal amounts may also enter *Sinapis* from a dry surface deposit by gaseous diffusion through the stomata or by diffusive penetration of the epidermis following sublimation. Movement of the substance within a plant is slight and appears to be mainly by diffusion. The selective action of 3:5-dinitro-*o*-cresol as a weed-killer is discussed in the light of these results.

Poisons or nutrients are frequently applied to foliage, but the processes by which they enter leaves have been little investigated. The use of 3:5-dinitro-*o*-cresol (referred to below as D.N.C.) as a selective weed-killer has been described by Robbins, Crafts & Raynor (1942) and by Blackman (1944, 1945). Weed-infested crops are usually sprayed with D.N.C. (up to 1% strength) as an aqueous suspension of the free acid or a solution of the sodium or ammonium salt. On drying, the spray leaves a residue of solid D.N.C., and, after 1–5 days, plants susceptible to the substance, e.g. *S. arvensis*, wither and die, whereas cereals remain relatively unaffected.

METHODS

D.N.C. preparations were made up from a stock suspension standardized against titanous sulphate (Wain, 1942). To control hydrogen ion concentration, sodium acetate at a final concentration of 0·5% was used as a buffer, pH being adjusted to the required value with hydrochloric acid. Acetate buffers alone are only slightly toxic to the test plants used.

Two methods have been used for the evaluation of the toxicity of D.N.C., using *S. arvensis* plants in the rosette stage, i.e. with cotyledons unwithered and two to five expanded foliage leaves. In the first method, samples of from three to five plants, the roots of which had been cut off under water, were immersed, with intermittent agitation, in the solution for a fixed time at a constant temperature. The plants were then washed in tap water, all damaged or unhealthy leaves removed, and about 1 cm. of the stem was clipped off to remove any solution in the base. The

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plants were afterwards stored in a cool greenhouse with their stems in water, and after 48 hr. the mature leaves were dissected into dead and living portions. From the dry weights of these two fractions a figure for percentage kill was obtained.

From a series of such determinations, made with varying immersion times or concentrations, estimates of median lethal doses have been made by means of linear regressions of kill expressed as probits on the logarithm of the dose, using the method for quantitative responses described by Finney (1947, pp. 183 *et seq.*). In determining weighting coefficients the variance of the responses at each dosage level have been assumed equal, an assumption substantially correct over the range covered. Since individuals are not being dealt with, the number of test subjects has been taken as unity in computing the weighting coefficient, the amount of plant material tested at each dosage level having been kept approximately constant in any one experiment. Analysis of variance has shown no statistically significant departure from the fitted curves (cf. Table 12).

In the second ('spraying') method, the solution or suspension was applied to plants in a cool greenhouse, using a hand 'atomizer', at 107·5 ml./m.^{2} (100 gal./acre). The plots were 1 ft. square and each contained twenty to thirty plants. The living and the dead plants were counted 48 hr. after treatment. A graded and replicated series of tests was made and the data analysed by the usual probit method (Finney, 1947).

Advancing contact angles were measured as described by Fogg (1947).

The penetration of D.N.C. into leaves

D.N.C. deposited on the surface of a leaf may reach the interior in three ways: (1) entry as solution or suspension through the stomata; (2) diffusion through the cuticle and epidermal walls; (3) diffusion as a gas through the stomata.

(1) *Entry as solution or suspension through the stomata*

Wetzel (1924) and Ziegenspeck (1942) state that injection by aqueous solutions through stomata takes place only under abnormal conditions. Åslander (1927) concluded that sulphuric acid enters *Sinapis* leaves *via* the stomata and also generally through the epidermis, but Crafts (1933) has criticized his methods and has shown that sulphuric acid does not enter through the stomata of sprayed *Convolvulus* leaves. Silversides (1940) reached a similar conclusion regarding the entry of cupric nitrate solution into *Taraxacum*. These workers based their conclusions on observations of leaf sections, a method that yields somewhat ambiguous results.

When injection of the sub-stomatal chamber does occur, it is readily visible under a direct-illumination microscope. No evidence of such injection with D.N.C. solutions has been observed using this technique. This finding was confirmed by experiments with epidermis stripped from leaves, in which the majority of stomata, although wide open, remained uninjected on immersion.

(2) *Diffusion through the cuticle and epidermal walls*

As there is no penetration through the stomata, it is evident that the toxic material, diffusing from aqueous solution, must be able to penetrate the epidermis. In the laboratory, a plant of *Sinapis* may absorb a lethal dose from a 0·01 % solution of D.N.C. in about 4 min., but under field conditions the spray liquid, a more concentrated solution of D.N.C., generally remains unevaporated on the plant for a much longer period.

Assuming that the process of penetration of D.N.C. is one of simple diffusion, the amount Q entering through an area A in time t at a given temperature will be related to the permeability P of the epidermis towards D.N.C., its thickness d, and the concentration gradient c of the substance between its two sides, according to the expression

$$Q = PAtc/d. \qquad (1)$$

The aim of spraying should be to make Q as large as possible by ensuring the maximum values for A, t and c, consistent with economy of effort and materials. However, the entities represented by these symbols are by no means simple and some consideration of their nature is necessary.

Direct tests of the validity of expression (1) are difficult. Q is small, a dilute solution of D.N.C. showing no appreciable change in concentration when a lethal dose is absorbed from it by a relatively large bulk of plant tissue. For example, after 20 g. of leaves of *Taraxacum officinale* had been immersed 2 hr. in 250 ml. of a 0·0006 g./100 ml. solution of D.N.C. no change in concentration of the solution could be detected by a colorimetric method. Since the sensitivity of this method was approximately 5 %, the amount of D.N.C. absorbed could not have been greater than 0·0037 mg./g. fresh tissue. It has not been found possible to extract D.N.C. in measurable quantities from treated plant tissue and therefore only indirect methods for estimating Q can be used.

Time. The median lethal concentration of D.N.C. has been determined for different periods of immersion of *Sinapis* (Tables 1 and 2). If equation (1) is obeyed, the product ct of the median lethal concentration and immersion time should be constant if Q, P, A and d remain constant. The fourth column of Table 2 shows that ct does tend to remain constant when t is varied sixteen-fold. The last column shows that variation in ct is mainly within the limits of experimental error, and that as far as time and concentration are concerned, equation (1) is approximately true.

Under field conditions the time available for penetration from solution is limited by evaporation. One way of prolonging this period is to add a hygroscopic substance to the spray liquid (Hopp & Linder, 1946). In this investigation, glycerol, calcium chloride and molasses have been found effective. Although additions of molasses produced a regular increase in damage to *Sinapis* in spray tests (Table 3), an immersion experiment showed that 10% molasses produced no statistically significant change in the median lethal immersion time for a D.N.C. solution (Table 4).

The effect thus appears to be on the distribution or duration of the spray liquid on the surface of the plant.

TABLE 1. *The relation of percentage kill of* Sinapis *leaves to time of immersion and concentration, using* D.N.C. *solutions at* 20° C. *and* pH 4·4

Concentration	Immersion time (min.)				
%	2·5	5·0	10·0	20·0	40·0
0·020	74·4	77·5	96·2	94·5	98·3
0·010	45·9	46·1	82·5	96·6	94·4
0·0050	6·25	25·0	62·5	86·5	85·2
0·0025	36·3	22·5	40·0	59·4	57·6
0·00125	11·0	9·08	36·1	22·0	30·0

TABLE 2. *The relation of median lethal concentration to immersion time, calculated from data in Table* 1

t = immersion time (min.), c = g. D.N.C./100 ml.

t	c (log units)	c	ct	log ct
2·5	1·043 ± 0·190	0·0110	0·0275	1·441 ± 0·190
5·0	0·985 ± 0·067	0·0097	0·0483	1·684 ± 0·067
10·0	0·455 ± 0·064	0·00285	0·0285	1·455 ± 0·064
20·0	0·338 ± 0·026	0·00218	0·0436	1·639 ± 0·026
40·0	0·308 ± 0·007	0·00203	0·0812	1·910 ± 0·007

TABLE 3. *Results of spraying test on* Sinapis *using molasses added to* 0·05% D.N.C. *solution buffered at* pH 4·6

% molasses (v/v)	0	1·0	2·5	5·0	10·0
% kill (mean of 2 replicates)	67·5	74·0	78·5	90·5	100

TABLE 4. *Results of immersion test on* Sinapis *using* 10% *molasses in* 0·01% D.N.C. *solution at* pH 4·6

Immersion time (sec.)	Percentage kill	
	Control	+molasses
60	0	0
120	30·2	17·9
240	74·9	69·8
480	100	100
Median lethal immersion time	165	169

The relative dosage value of the control with respect to the test solution = 0·055 ± 0·0257.

Absorption from, or loss of water to, the air by the spray solution will take place until equilibrium is reached. The amount of hygroscopic substance added will thus affect the final volume and consequently the area of cover of the solution rather than its rate of drying, which will be indefinitely prolonged.

Concentration. Tables 1 and 2 also show that the rate of entry of D.N.C. into leaves is proportional to its concentration on the outside of the epidermis.

Krahl & Clowes (1938*a*, *b*) have shown that nitro-phenols penetrate the eggs of *Arbacia* only in the unionized form, but the cuticle and epidermal walls of a leaf are non-living structures to which these findings do not necessarily apply. Median lethal immersion times were determined for solutions containing the same total amount of D.N.C. but of different pH values and, therefore, containing different proportions of the dissociated and undissociated forms (Tables 5 and 6). The concentrations of the two forms have been calculated using the value for pK′ for D.N.C. of 4·4 (Krahl & Clowes, 1938*a*). Table 6 shows that the product $t[HP]$ is constant within the limits of experimental error, whereas there is a nearly fortyfold and statistically significant variation in $t[P^-]$.

TABLE 5. *The relation of percentage kill of* Sinapis *leaves to time of immersion in* D.N.C. *solutions buffered at different* pH *values*

D.N.C. concentration 0·010 %. Temperature 20° C.

Immersion time (min.)	pH 3·8	4·3	4·8	5·3	5·8
1	59·9	62·6	30·9	45·2	24·8
2	74·9	50·0	40·1	36·3	20·0
4	80·0	77·6	69·8	50·0	38·2
8	87·5	80·0	74·9	69·8	59·9
16	100	90·8	91·0	66·6	71·2

TABLE 6. *The relation of median lethal immersion time t, to concentration of undissociated* D.N.C. $[HP]$, *and of dissociated* D.N.C. $[P^-]$, *both expressed as fractions of* $M \times 10^{-3}$.

Calculations from data of Table 5.

pH	log t	t	$[HP]$	$[P^-]$	$t[HP]$	$t[P^-]$	log $t[HP]$
3·8	$\bar{1}\cdot766 \pm 0\cdot115$	0·58	0·403	0·102	0·234	0·059	$\bar{1}\cdot369 \pm 0\cdot115$
4·3	$\bar{1}\cdot875 \pm 0\cdot290$	0·75	0·281	0·224	0·211	0·168	$\bar{1}\cdot324 \pm 0\cdot290$
4·8	$0\cdot372 \pm 0\cdot053$	2·35	0·144	0·361	0·338	0·848	$\bar{1}\cdot529 \pm 0\cdot053$
5·3	$0\cdot464 \pm 0\cdot160$	2·91	0·056	0·449	0·164	1·306	$\bar{1}\cdot215 \pm 0\cdot160$
5·8	$0\cdot776 \pm 0\cdot076$	5·97	0·0193	0·4857	0·115	2·900	$\bar{1}\cdot061 \pm 0\cdot076$

Since the product of concentration and time should remain constant in this experiment if expression (1) applies, this result seems to show that undissociated D.N.C. is the penetrating form. If both dissociated and undissociated D.N.C. penetrate the epidermis the former may be ineffective because the living cells are impermeable to it. But absorption of the undissociated form only, from the intercellular solution, will result in recombination of hydrogen ion and anion to restore equilibrium. The total amount of D.N.C. present inside the plant is thus potentially capable of toxic action, and if the anion penetrated the epidermis at approximately the same rate as the undissociated D.N.C., the toxic effect would depend more on the total external concentration than on pH.

When a suspension of D.N.C. is used, the undissolved solid exerts an appreciable buffering effect since removal of hydrogen ion is compensated for by further

dissociation of D.N.C., the concentration of the undissociated form being maintained by solution of the solid.

When, as in practice, the alkaline solutions of the sodium or ammonium salts are used, then, notwithstanding the greater concentrations attainable, that of the active undissociated form is much smaller than in acid solution. A solution of the sodium salt containing 0·5% of D.N.C. (0·0252M) at pH 7·0 contains a concentration of 0·000063 M of the undissociated form; the corresponding concentration in a saturated solution of D.N.C. in a sodium acetate buffer at 20° C. is approximately 0·0012 M at pH 4·4. Whether the kation is sodium or ammonium has little effect on the penetration of D.N.C. into leaves from solution (Table 7).

TABLE 7. *Results of immersion test on* Sinapis *using sodium and ammonium dinitro-o-cresylates at* 0·25% D.N.C. *and* pH 6·5

Immersion time (min.)	Percentage kill	
	Sodium	Ammonium
1	32·4	23·8
2	41·4	46·7
4	82·5	81·7
8	70·0	81·0
16	97·5	92·0
Median lethal immersion time	125·8 sec.	121·0 sec.

Relative dosage value of the ammonium salt with respect to the sodium salt = 0·0170 ± 0·122.

Area. Expression (1) requires that the amount of D.N.C. penetrating the epidermis from solution should be directly proportional to the area over which diffusion is possible. This area is difficult to measure and depends on the spray solution, the plant surfaces, and the manner of application. The proportion of the surface of a sprayed plant in contact with drops depends on the contact angle of the solution against the epidermal surface (Fogg, 1947). The advancing contact angles of aqueous D.N.C. preparations are not appreciably different from those of clean water, e.g. on leaves of *Triticum* a 0·3% D.N.C. suspension had a mean contact angle of 136·2 ± 1·24°, whereas that for water was 136·1 ± 1·74°.

The actual and the superficial areas of contact are not necessarily the same, since the epidermis may be corrugated or hairy. Where the epidermis is not readily wetted, air films are retained beneath drops to an extent depending on its corrugation. Rudolph (1925) has recorded that, on wilting, leaves become more resistant to toxic solutions. Wilted leaves of *Sinapis* and of *Taraxacum officinale* have been found to be less damaged by immersion in a D.N.C. solution than are otherwise similar fresh leaves. That this is due to decreased penetration of D.N.C. into the wilted leaf rather than to decreased susceptibility of its cells was shown by injection with the same solution, the wilted leaves in this case being more damaged than the controls. It appears to result from a decreased actual area of contact between

solution and leaf surface depending on an increase in corrugation of leaf surfaces occurring during wilting (Fogg, 1947).

Observations of a *Sinapis* leaf covered with D.N.C. solution made with a direct illumination microscope showed that as evaporation proceeded the drops decreased in depth but only slightly in area, until eventually the film broke up leaving no appreciable liquid residue.

The effects of suspended solids. The effect on the area of contact of suspended solid matter was investigated by adding Indian ink to spray solutions, when an estimate of the cover after drying could be made with a low-power microscope. Whereas a saturated solution of D.N.C. applied to *Sinapis* leaves with an 'atomizer' covered $23{\cdot}5 \pm 2{\cdot}23\%$ of their upper surfaces, a $0{\cdot}5\%$ suspension applied in the same way to a similar sample of leaves gave a cover of $33{\cdot}1 \pm 1{\cdot}63\%$, the difference being significant at $P < 0{\cdot}01 > 0{\cdot}001$.

In this mechanical role as a spreader, solid D.N.C. may be replaced by powdered talc or silica. The proportion of surface covered increases with the content of suspended solid, being proportional to the logarithm of the latter (Table 8). Weight

TABLE 8. *Effect of talc (particle diameter c.* 7 μ) *on cover of* 0·05% D.N.C. *solution on* Sinapis *leaves*

% talc (w/v)	0	1	3	5	7	9
% cover (mean of 6)	19	35	43	48	52	54

for weight a powder with a smaller average particle size has been found more effective than a coarser one. The suspended solid has no appreciable effect on advancing contact angle. Thus the mean contact angle of a 0·4% D.N.C. suspension on a paraffin surface was $86{\cdot}65 \pm 0{\cdot}87°$ and that of its filtrate $88{\cdot}74 \pm 1{\cdot}97°$, indicating that a solid spreader does not act by increasing the adhesion of the spray liquid for the leaf surface but by hindering the contraction of the drops. The solution remains spread as menisci between the accumulations of particles rather than rounding off into droplets. There is some tendency for the solid to collect in the valleys between epidermal cells, regions that are perhaps more permeable to dissolved substances than other areas of the epidermis (p. 323). Evaporation takes place in much the same way as described for D.N.C. solution.

The increase in area of cover of a D.N.C. solution produced by talc is accompanied by a corresponding increase in toxic effect (Table 9). If the amount of D.N.C. entering the plant is directly proportional to the area of penetration (equation (1)) then the toxicity should show the same relationship to area of cover as it does to dose, i.e. probit kill plotted against log area of cover should give a straight line. The expected kills derived from the regression computed on this assumption (Table 9, column 6) are in reasonable agreement with the observed values and an analysis of variance (Table 10) shows that the fitted regression accounts for most of the variation in the data, thus agreeing with the hypothesis that Q is proportional to A.

Deviation from the fitted curve, however, just reaches statistical significance at the $P=0{\cdot}05$ level, indicating that the relationship is perhaps more complex than this.

TABLE 9. *Effect of talc on cover and toxicity of* 0·05 % D.N.C. *on* Sinapis *leaves: toxicity determinations on comparable plants by the spraying method. Twenty-five plants per test*

% talc (w/v)	% cover on leaf	log % cover (x)	% plants killed		% kill calculated
			A	*B*	
0	16·2	1·209	15	14	16·4
0·25	24·4	1·387	64	69	60·8
0·50	28·6	1·456	64	78	77·6
0·75	31·8	1·502	91	98	86·0
1·00	35·4	1·549	87	89	92·1

$$Y=5{\cdot}414+7{\cdot}03\,(x-1{\cdot}407)$$

TABLE 10. *Analysis of variance of the data in Table* 9

The sum of squares for deviation from the regression, $S\,(\bar{u}-U)^2$, is formed from the differences between the observed kills and those estimated from the fitted regression line.

	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Within arrays	1	72·9	72·9	—
Between arrays:				
Regression	1	7601·96	7601·96	—
Deviations	3	337·44	112·48	6·97*
Error	4	64·6	16·15	
	9	8076·90		

* P approximately $=0{\cdot}05$

TABLE 11. *Result of immersion test on* Sinapis *leaves using suspended solids in saturated solution of* D.N.C. (0·057 g./100 ml.)

A, control; *B*, 0·5 % solid D.N.C.; *C*, 0·5 % powdered talc.

Time (sec.)	Percentage kill		
	A	*B*	*C*
19	25·7	34·3	57·7
38	51·8	50·0	50·0
75	51·5	43·7	60·8
150	75·0	78·6	72·7
300	77·8	84·6	80·0
600	89·5	93·3	78·9

Median lethal immersion time = 39·81 sec. with fiducial limits at the 95 % level of 28·4 and 55·9 sec.

It was shown by an immersion test (Table 11) that suspended solid promotes the penetration of D.N.C., only when the solution is applied as a spray. Table 12 shows that the presence of suspended solid D.N.C. or talc caused no statistically significant changes in the toxicity of a saturated D.N.C. solution.

Permeability. It is well known that the epidermis of most plants, although containing layers consisting largely of lipides, is permeable to a limited extent to

water and other substances. Rudolph (1925) has given qualitative evidence that hydrochloric acid does not penetrate to any appreciable extent in the form of vapour through the stomata and it seems unlikely that the proportion of D.N.C. entering in this way can be important. Hypostomatous leaves of *Tilia europaea* or *Urtica dioica*, treated with D.N.C. solution on the upper surface only, are affected to the same extent as similar leaves treated on the lower surface only, so that, in some cases at least, penetration is through the epidermis itself rather than the stomata.

TABLE 12. *Analysis of variance of the data in Table* 11

The sum of squares for deviation from the regression, $S(\bar{u}-U)^2$, is formed from the differences between the observed kills and those calculated from the probit equation.

	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Between arrays:				
Regression	1	5376·76	5376·76	
Deviations	4	291·48	72·87	1·07*
Error	10	781·12	78·11	
Within arrays	2	69·28	34·64	2·25*
	17	6518·64		

* P in both cases is greater than 0·20

Rudolph (1925) has put forward much evidence to show that the passage of water and water-soluble substances through the cuticle and outermost walls of the epidermis is largely confined to regions over the anticlinal walls. More recently, Rouschal & Strugger (1940) have shown that excretion of salts from the interior of leaves is similarly restricted to particular areas. As Rudolph has pointed out, this is due to the fact that, in the outer epidermal walls, lamellae containing different proportions of lipide and water-imbibed constituents run parallel with the leaf surface, except at the anticlinal walls where they dip inwards at right angles to this plane. There is no ground for supposing that the cuticle is other than uniformly permeable over all its area (Rudolph, 1925), nor is there direct evidence of sub-microscopical pores such as postulated by Rouschal & Strugger (1940). Electron microscope studies on the cuticle from *Antirrhinum* (Watson, 1943) show it to be a structureless film in young leaves; in older leaves it is definitely fibrous but no holes have been detected.

Unless a superficial deposit of wax is present, the cuticle, a lipide membrane, is the first barrier to be penetrated by D.N.C. diffusing from an aqueous solution on the leaf surface. Undissociated D.N.C. is readily soluble in lipides, whereas its sodium and ammonium salts are not. It has already been shown (p. 319) that it is only the undissociated form that penetrates the epidermis of *Sinapis*. It is to be expected that penetration will occur uniformly over all the area of the cuticle in contact with the solution. In the cutinized layers of the epidermis diffusion of D.N.C. may occur in either the lipide or aqueous phase and in addition there may be transport of D.N.C. along the water-lipide interfaces. Rudolph's findings suggest that penetration of D.N.C. is greatest opposite the anticlinal walls. These channels are probably

fed by lateral movement of D.N.C. so that diffusion through the cuticle continues more or less uniformly over all its area in spite of the restriction on its movement in the layers below. This is perhaps an important point, since if the epidermal surface is incompletely wetted those regions over the anticlinal walls, being below the general surface level, will not be in contact with the solution. Until they are killed, the protoplasts of the epidermis are probably nearly impermeable to D.N.C. and the anticlinal walls are apparently the main channels of entry into the mesophyll.

That the main resistance to the entry of D.N.C. into a leaf is offered by the cuticle is indicated by the effect of temperature on the process. The temperature coefficient (Q_{10}) of P has been determined by the immersion technique (Tables 13 and 14).

TABLE 13. *Penetration of* D.N.C. *into* Sinapis *leaves at various temperatures as determined by the immersion method*

0·01 % D.N.C. solution buffered at pH 4·5. After immersion all plants were stored at *c.* 20° C.

Immersion time (min.)	Percentage kill			
	0° C.	10° C.	20° C.	30° C.
0·25	—	—	0	50
0·50	—	0	71	85
1·0	0	28	83	100
2·0	—	71	100	100
4·0	62·5	78	100	100
8·0	78	100	—	—
16·0	100	100	—	—
32·0	100	100	—	—

TABLE 14. *The relation of the permeability constant, P, of the epidermis of* Sinapis *leaves for* D.N.C. *to temperature*

Calculations from data in Table 13. Median lethal immersion time, t, in minutes.

T (°K.)	t (log units)	t	$1/t$ ($\propto P$)
273	1·450 ± 0·046	3·52	0·284
283	1·095 ± 0·048	1·57	0·642
293	0·561 ± 0·081	0·455	2·20
303	0·336 ± 0·017	0·271	3·69

Samples of *Sinapis* were treated with D.N.C. solutions at four different temperatures and afterwards stored all at the same temperature. D.N.C. penetration varied with immersion temperature, but subsequent exposure of the cells to the toxic substance was at the same temperature. The damage resulting from a given dose will thus have been the same providing that the pretreatment at different temperatures did not markedly affect the susceptibility of the plants towards D.N.C. It will be seen that the median lethal immersion time decreased with rise in temperature, indicating an increased rate of penetration. The Q_{10} is approximately 2·6. If $\log P/\sqrt{T}$ is plotted against $1/T$ the points lie close to a straight line (Fig. 1), an indication that the structure of the membrane concerned does not vary significantly with temperature

and that, as far as penetration of D.N.C. is concerned, it approaches homogeneity (Davson & Danielli, 1943, p. 315).

Thickness of epidermis. Rudolph (1925) found that in general the time required to kill leaves by immersion in sulphuric acid increases with thickness of cuticle. It seems reasonable to suppose that the same applies to the penetration of D.N.C. through the epidermis. Observations on a number of species support this conclusion, but although d varies from species to species the composition and permeability of the membrane also vary so that precise comparisons are difficult.

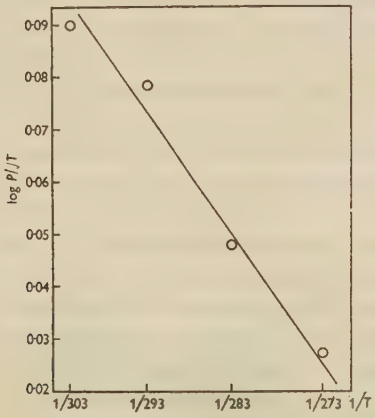

Fig. 1

Discussion. These results are in accordance with the hypothesis that penetration of D.N.C. into a leaf from aqueous solution is a process of diffusion through a membrane, complicated mainly by the irregularities in structure of the epidermis. The concentration gradient of D.N.C. between the epidermal surface and the mesophyll is not expressible in simple terms since the membrane is composite, and adsorption, chemical interaction between D.N.C. and constituents of the intercellular sap, and absorption by cells may occur to greater or lesser extents. No experimental data on this are available. Modes of entry other than simple diffusion are possible, e.g. movement of D.N.C. into a leaf may be assisted or opposed by water movements, which might occur if the spray solution is of an osmotic concentration markedly different from that of the leaf tissues.

(3) *Entry of* D.N.C. *by gaseous diffusion*

Experiments in which D.N.C. spray residues have been washed off plants as soon as they dried have shown that the solid residue is necessary for the full toxic effect. With susceptible material such as young *Sinapis* a complete kill can be obtained when the spray residue has been washed off in this manner, but with the more resistant mature plants of *Raphanus raphanistrum* the same treatment results in

a marked decrease in damage. Furthermore, in several experiments, solid D.N.C. dusted on to plants has produced a toxic effect, although always less than that produced by an equivalent amount of D.N.C. in solution. Two mechanisms seem to be possible for the penetration into plants of D.N.C. from dry spray residues:

(1) Since the cuticle is water-imbibed it is possible for D.N.C. to go into solution at the points of contact and diffuse as from an aqueous solution.

(2) Since appreciable quantities of D.N.C. may sublime at ordinary air temperatures it is probable that it penetrates as a gas. The rate of sublimation of D.N.C. in still air at 20° C. was found to be 0·0238 mg./day/cm.^{2}.

When *Sinapis* was kept in a closed container at 25° C. with solid D.N.C., all contact between plants and the latter being rigorously excluded, the plants were killed in 24 hr. and traces of D.N.C. were noticed on them, showing that sublimation had taken place. In another experiment plants sprayed with a D.N.C. suspension were placed under a bell-jar together with unsprayed plants. Contact of the untreated with the sprayed plants was prevented but, nevertheless, they died in the course of 48 hr.

In a further experiment air was allowed to take up D.N.C. vapour by bubbling it through the molten substance, excess being afterwards condensed out at 25° C., and passed at 0·25 l./min. into a large desiccator containing *Sinapis* plants at the same temperature. The median lethal dosage time was 14·03 min. with fiducial limits of 8·6 and 22·9 min. at the 95% probability level (Table 15). This result is not strictly comparable with the results of the immersion tests, but it is clear that a longer exposure is required to give a kill under these conditions. An approximate figure for the median lethal dosage time at 19° C. is 10 hr. (Table 16).

Table 15. *Lethal effect on* Sinapis *of air saturated with* D.N.C. *vapour at* 25° C.

Kill corrected for mortality in controls (4·8%).

Time (min.)	4	8	16	32
% kill	15·2	22·3	52·1	89·0

Table 16. *Toxic effect of vapour of* D.N.C. *and its salts on* Sinapis

Exposure (hr.)	Percentage kill			
	D.N.C.	Ammonium salt	Sodium salt	Control
3·5	9·8	6·9	4·6	1·9
16·0	—	51·2	4·3	0
20·7	82·2	80·6	0·0	2·6
Approximate median lethal dosage time	10	13	∞	∞

Since under favourable conditions a D.N.C. deposit on foliage persists for over 4 days, there may be ample opportunity for penetration of sufficient D.N.C. vapour to exert considerable damage. Rain has to be heavy or prolonged to remove the

D.N.C. deposit entirely: in its absence the solid D.N.C. disappears slowly by sublimation. Temperature and air movement have most effect on the penetration from the vapour phase. No determination of the relation of vapour pressure of D.N.C. to temperature has been made, but experience suggests that it becomes negligibly small at about 10° C. In an experiment with *Anagallis arvensis* it was found that an air movement of 1·6 m./sec. reduced the kill produced by solid D.N.C. on the foliage to about 18% as compared with 30% for control plants in still air.

Results obtained with *Sinapis* (Table 16) suggest that the ammonium salt is about as volatile as the free acid but that the sodium salt is much less volatile. Glass plates were coated with the solid to be tested and tubes containing the test plants were placed on them and covered with a bell-jar.

The mechanism of penetration via the vapour phase. D.N.C. vapour will enter a leaf through open stomata, and it is to be expected that appreciable amounts will penetrate in this way. An alternative path is by solution of the vapour in the aqueous phase of the cuticle followed by diffusion through the epidermis. This is evidently important in some species at least, because hypostomatous leaves of *Urtica dioca* with the lower surface covered with petroleum jelly are killed by D.N.C. vapour as readily as leaves covered on the upper surface with this substance. The ability of D.N.C. to spread along certain interfaces is shown by the outward spread of D.N.C. staining from a crystal placed on filter-paper. There was no perceptible difference in the diameter of the stained patches after 24 hr. on filter-paper in contact with solid D.N.C. and on filter-paper 2 mm. away, and such spreading is very much reduced below 70% R.H. These facts suggest gaseous diffusion followed by solution at the surface of a hygroscopic solid. Since the vapour pressure of D.N.C. is lowered on solution, distillation from the solid will occur until the adjacent surfaces are saturated. In this fashion D.N.C. may spread without the bulk of the gaseous phase becoming saturated with vapour. Thus the water-imbibed cuticle will in a few hours become saturated with D.N.C. which may then diffuse through the epidermis. Penetration into the interior of the leaf may also occur by spreading over the walls of the stomatal pores.

(4) *Transport of* D.N.C. *within the plant*

Translocation of D.N.C. from organ to organ is not appreciable: D.N.C. applied to one leaf does not move internally in quantities sufficient to affect other leaves on the same plant. When D.N.C. is applied to a small incision on a leaf of *Vicia faba* a circular necrotic area is formed after 2 days, but leaves without an incision show only a small necrotic patch immediately under the D.N.C., demonstrating that it spreads internally. The extent of the killed patches is independent of venation but the damaged area tends to extend towards the edge of the leaf. This internal spreading of D.N.C. is different from that of an injected dye (Roach, 1939) and appears to be mainly by diffusion in cell walls. The rate of advance was *c.* 1 mm. in 17 hr. in one experiment.

Discussion

It is shown that penetration of D.N.C. from a spray deposit into leaves takes place in two distinct phases. The relative importance of these depends on the weather: for example, the phase of diffusion from aqueous solution will be of most importance in damp cold weather whereas penetration *via* the vapour phase will predominate under hot dry conditions. The relative importance of the two phases will also vary from species to species.

The most important single factor affecting retention of a spray solution is its contact angle on the plant surface (Fogg, 1947), other factors such as hairiness or roughness of the plant surfaces being of subsidiary importance. On species resistant to D.N.C., water has a contact angle greater than 100°, but there is no correlation of resistance with hairiness. The contact angle of water is low on *Sinapis* leaves and high on cereal leaves (Fogg, 1947); accordingly, retention of aqueous sprays by the former is greater (Table 17). However, differences in retention do not account for all the differences in susceptibility between *Sinapis* and cereals, since an immersion treatment which gave 100% kill with *Sinapis* had no effect on *Triticum* or other cereals.

Table 17. *Retention by leaves of a* 0·4% D.N.C. *suspension 'atomized' at* 107·5 *ml./m.*^{2} *of soil surface*

D.N.C. estimated colorimetrically after washing off the leaves.

	No. of determinations	Mean retention (mg./cm.^{2})	Approx. advancing contact angle
Sinapis arvensis	7	0·0251 ± 0·0019	63°
Avena sativa	6	0·0018 ± 0·00016	130°
Hordeum sativum	1	0·0021	136°

Penetration of D.N.C. into cereal leaves may be less than that into *Sinapis* leaves because of a smaller area of contact between the spray drops and the epidermal surface, a smaller time of diffusion, a smaller permeability or a greater thickness of the epidermis. Fogg (1947) showed that the superficial area of contact of a spray deposit on *Triticum* leaves is smaller than the corresponding area for *Sinapis*. Furthermore, the actual area of contact is probably smaller in the former than in the latter owing to a greater corrugation of the epidermal surface (Fogg, 1947). But, in immersion trials in which cereal leaves are completely covered with D.N.C. solution plus a wetting agent, doses lethal for *Sinapis* have no effects on cereals.

Since the drops retained on *Triticum* are smaller than those on *Sinapis* leaves (Fogg, 1947) their surface area will be relatively larger, and hence evaporation will be more rapid. No reliable means of comparing the permeabilities towards D.N.C. of the epidermises of the two types has been worked out. *Sinapis* cuticle is *c.* 1·5 μ thick; that of cereals, 2·5–3·0 μ. All these considerations indicate that the rate of penetration of D.N.C. into cereal leaves in the first phase is much slower than that into *Sinapis* leaves.

During the second phase of penetration this disparity is perhaps less pronounced, since some D.N.C. may enter cereal leaves *via* the stomata even though little penetrates the epidermis from the vapour phase.

As the amount of D.N.C. received by, and penetrating into, a plant will depend on its surface area while its effect will depend on the dose per unit bulk of tissue, a species with a relatively small surface/volume ratio will be less damaged by the substance than one with a larger ratio, other things being equal. The ratio, surface area ($cm.^2$)/fresh weight (g.) for whole shoots was found to be as follows:

Sinapis, young plants, rosette stage	$30{\cdot}0 \pm 0{\cdot}49$
flowering stage	$21{\cdot}85 \pm 1{\cdot}25$
Triticum vulgare	$27{\cdot}7 \pm 0{\cdot}57$

Although statistically significant, these differences are not sufficient to account for the differences in susceptibility, nor are they always correlated with them.

There may be further factors, depending on internal conditions in a plant, which contribute towards the selective effect. If D.N.C. solution is infiltrated into *Triticum* leaves using the method of Weber (1927) they are very little damaged although *Sinapis* leaves treated similarly are readily killed. In another type of experiment shoots of *Hordeum sativum* and *Sinapis* were allowed to take up dilute D.N.C. solution through the cut end of the stem. Transpiration was followed potometrically and the time in the solution adjusted so that the two types of plant took up equal quantities of D.N.C. per unit fresh weight. *Sinapis* was killed by this treatment but no damage to the cereal could be detected (killed areas in cereal leaves were shown up by infiltration of the leaf with a solution of neutral red, which stained dead areas much more heavily than healthy tissues).

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ADDENDUM

In a recent discussion, Adam (*Trans. Faraday Soc.* 1948, in the press) has considered the effect of a constriction on the penetration of liquids through a pore. His theoretical treatment is applicable to the case of the usual type of stoma with cutinized ridges and shows clearly, in agreement with the results given above, that spontaneous entry is not to be expected, even when the stoma is wide open, unless liquids of very low surface tension are employed.

THE PERSISTENCE OF HYDROGEN CYANIDE IN RABBIT BURROWS

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(With 9 Text-figures)

Experiments were carried out in an artificial rabbit burrow to test the persistence of concentrations of hydrogen cyanide. The gas was evolved from two kinds of trade powders (Cymag and Cyanogas), placed in a heap just inside the mouth of the burrow, which was then sealed in with earth. Samples of the mixture inside the burrow were withdrawn from various points along its length at stated intervals, and the concentrations of hydrogen cyanide determined by a rapid and easy method, which is described, and plotted against time.

Data on the toxicity of hydrogen cyanide to rabbits are considered and the following critical points determined: 1 part in 1250 by volume (1 mg./l.) kills 50% of rabbits in 1 min.; 1 part in 5400 (0·22 mg./l.) kills in 18 min.; and 1 part in 7500 (0·16 mg./l.) is at the threshold of toxicity. Collapse, which is probably the most important point from a practical point of view, occurs in about a third of these times.

The persistence of HCN concentration resulting from the use of half an ounce (14·15 g.) of either Cymag or Cyanogas cannot be regarded as sufficient to guarantee good results.

With a dose of 1 oz. (28·3 g.) in summer a concentration of 1 part in 1250 is maintained for 34 hr. (Cyanogas) and for 48 hr. (Cymag), and a concentration of 1 part in 5400 for about 80 hr. by both materials. This means that a concentration which will kill rabbits in 1 min. can be maintained over the second night after gassing by either substance, but Cyanogas must be applied late in the day.

Under winter conditions a concentration of 1 part in 1250 is only reached by Cymag for a short period between 16 and 25 hr. after the start of the experiments; by Cyanogas not at all. On the other hand, a concentration of moderate toxicity (1 part in 5400) is maintained for about 120 hr. Probably a dose somewhat greater than 1 oz. would be an advantage in the winter.

In summer the initial rise in concentration is immediate: in winter there is a delay of some 10–15 hr. before a good concentration is built up. Under these conditions it will be better to gas rabbit warrens early in the day, or alternatively to scatter the powder slightly.

In general, the characteristic differences shown between Cymag and Cyanogas are due to the buffering effect produced on the evolution of hydrogen cyanide from Cymag by the increasing concentration in the surrounding air. Thus the curve has a typical flat-topped form, contrasting with the very high peak, quickly rising and falling, produced by Cyanogas.

Diffusion of the hydrogen cyanide down the burrow away from the source is very small in the summer (a sampling point 40 in. away from the source was consistently negative throughout the experiments), and only slightly more in the winter.

In 1940 A. D. Middleton carried out a series of tests on the practical efficiency of trade materials containing hydrogen cyanide for reducing and exterminating populations of the wild rabbit, *Oryctolagus cuniculus* (L.). These tests were made by gassing warrens in different ways and digging out the whole burrow systems to verify and compare the results. From these empirical data it was maintained

(Middleton, 1940) that the method of placing a heap of powder just inside the mouth of each burrow and sealing with turf or soil gave results as good as or better than blowing the powder throughout the warren with a pump and was simpler and cheaper.

The strategy behind these two methods differs considerably. Most of the trade cyanide powders act by evolving hydrogen cyanide when they are brought into contact with atmospheric moisture. When the powder is forced through the warren with a foot pump or a fan blower the aim is to fill every passage with finely distributed powder so that a high concentration of hydrogen cyanide is produced almost immediately. By this method the rabbits in the warren should be killed almost at once, wherever they happen to be lying. The main obstacles to achieving this result are the blind passages, which either act as air locks or are blocked by a rabbit sitting in the entrance.

With the so-called 'spooning' method the aim is to create a lethal zone of hydrogen cyanide just inside each burrow mouth, which will persist for 24 hr. or more. Thus the rabbits will only be killed as they become active towards evening and come up to scratch through the blocked entrances. The persistence is obtained by placing the powder within the burrow in a compact heap so that the evolution of hydrogen cyanide is retarded.

The experiments described in the present paper were designed to show how long a lethal concentration would remain under stated conditions. A number of variable factors, such as temperature, relative humidity, surface area of the powder exposed, size of dose for each burrow and so on, all needed investigation before a sound practical technique for securing good results could be achieved.

Materials tested

Two types of cyanide powder were tested. The first is made by Imperial Chemical Industries and marketed under the name of Cymag. It is a mixture of sodium cyanide and magnesium sulphate, and 1 g. of the powder contains 0·202 g. of hydrogen cyanide. The second is made by the American Cyanamid Co. and distributed in Great Britain by Messrs Geo. Monro Ltd. under the name of Cyanogas. It consists of finely ground calcium cyanide, 1 g. of which contains 0·251 g. of hydrogen cyanide. A variant of this, Cyanogas G, which is somewhat less finely ground, was also tested. At the time of writing only Cymag is available in Great Britain.

The characteristics of these two products are shown by the described experiments. At the moment it is sufficient to note that the evolution of hydrogen cyanide is much more rapid from Cyanogas than from Cymag. Not only is there a critical relative humidity* below which the evolution from Cymag is almost indefinitely slowed down, but when the concentration of hydrogen cyanide surrounding the

* Data kindly supplied by I.C.I. Ltd. show that this factor will rarely operate in Great Britain, since the critical figure varies from 77% R.H. at 5° C. to 69% R.H. at 20° C.

powder reaches a certain level this automatically reduces the rate of evolution of the gas.

Methods used

For the purpose of these experiments an artificial rabbit burrow was excavated in stiffish loam with Valley Gravel subsoil adjacent to the University Museum, Oxford (Fig. 1). The sampling points from which the air and hydrogen cyanide mixture was withdrawn at stated intervals for determination were arranged by setting metal tubes into the surface of the ground and consolidating them into the earth with putty. These tubes were kept tightly corked.

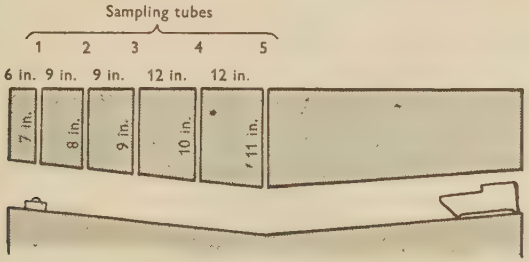

Fig. 1. Cross-section of artificial burrow, showing dimensions and positions of sampling tubes, of powder at the left-hand end, and of thermo-hygrograph at right-hand end of the burrow.

Throughout each experiment temperature and relative humidity records inside the burrow were obtained by using a Casella thermo-hygrograph.

The burrow was first aerated by leaving both ends open for 24 hr. The thermo-hygrograph was then placed in one end of the burrow, which was sealed with a turf and loose soil firmly stamped down. At the other end, a small tin tray containing first a layer of soil and then the relevant dose of powder was placed directly under the first sampling tube. This end was then also sealed. The powder was placed in as compact a heap as possible; a 0·5 oz. (14·15 g.) dose covered about 2·5 sq.in., a 1 oz. dose (28·3 g.) about 4 sq.in.

Two sources of error were checked during the course of these experiments. The amount of hydrogen cyanide remaining on the walls of the burrow after 24 hr. aeration was checked by removing 4 g. of soil from near the tray, shaking up with 10 ml. of distilled water, filtering and adding 3 ml. of standard indicator solution (for full details of the colorimetric method of estimation used, see Appendix). No colour change was noted in 48 hr., so this source of error was deemed to be negligible.

Another possible source of error was the fact that each experiment involved about fifty estimations, for which 6 l. of air and hydrogen cyanide mixture were withdrawn from the burrow, the total capacity of which was 31 l. In fact the experiments show

that this effect is not serious at the higher concentrations: only where the concentrations are low, either towards the end of the test or at the end of the concentration gradient—sampling point 4 (30 in. away from the source of the gas)—are there noticeable fluctuations due to this cause (see, for example, Fig. 3). In any case the total amount of hydrogen cyanide removed from the burrow for fifty samples could not have been more than 12 mg. out of a total of as many grams.

Results

Summer experiments

The experiments fall into two groups. The first four were done during the summer and comprise tests done with Cymag and Cyanogas at doses of 1 and 0·5 oz.; the last two were done under winter conditions and were replicas of the summer experiments with a 1 oz. dose. The results are shown in Figs. 2–7; tables giving the full data are deposited with the Bureau of Animal Population at Oxford.

Exp. A (Fig. 2). In this and the next three experiments a start was made early in the morning, so that at least four samples could be taken during the first 12 hr.; after that about three samples per day were taken.

The most striking result is the concentration gradient that was formed owing to the tendency of the hydrogen cyanide to collect at the higher end of the burrow. Sampling point 5, 42 in. from the source of the gas, was used throughout the experiment, but gave a consistently negative result, and so is omitted from the figure. At sampling point 4 (30 in. from the source of the gas), there was no trace until 12 hr. after the experiment had started and even then it never rose to more than 1 part in 5400 (0·22 mg./l.), which would take a long time to kill a rabbit (see p. 341). At sampling point 3, which was 18 in. away from the source, the lag in building up a concentration was quite marked, and the peak never reached as high a level as at points 1 and 2. These last, no. 1, directly over the source, and no. 2, only 9 in. away, showed very little difference throughout the experiment.

The general nature of the curve is interesting. After the initial sharp rise in concentration an equilibrium is established by the slowing down of evolution of the gas (illustrated by the bluntness of the peak of the curve), and a high level of hydrogen cyanide (above 1 part in 1250 or 1·00 mg./l.) is maintained for nearly 50 hr. This only refers to sampling points 1 and 2, i.e. within 9 in. of the source of the gas. The concentration continues to fall rapidly for the next 10 hr. and then the curve flattens out reaching the level of 1 part in 5400 (0·22 mg./l.) by about 82 hr. (just beyond the limit of the observations).

Piling up the powder to expose as small a surface area as possible does not prevent a satisfactory initial rise in the concentration. Within half an hour this is sufficient to kill rapidly any rabbits that come into the gas zone.

Relative humidity was constant at 86 % throughout the experiment. The temperature gradually increased (the day maximum rose by 2·5° C., the night minimum by 1·5° C.), but probably not sufficiently to vitiate any of the results.

Exp. B (Fig. 3). This was completely comparable to Exp. A, but carried out with 1 oz. of Cyanogas instead of Cymag. Relative humidity was the same; temperature was slightly higher, starting at 17·5° C. instead of 15·5° C. and maintaining a regular fluctuation of 1·0–1·5° C. between day maxima and night minima. A preliminary check was made to ensure that acetylene, which is given off in small amounts by Cyanogas, does not affect the colour reactions upon which the estimations are based.

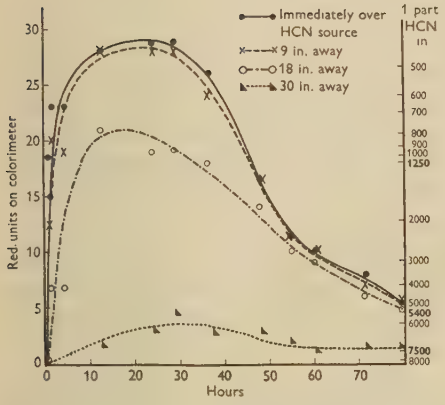

Fig. 2. Persistence of hydrogen cyanide concentrations with 1 oz. (28·3 g.) Cymag under summer conditions. Curves drawn freehand through the points shown. Critical levels (1 part in 1250 lethal to 50% of rabbits in 1 min.; 1 part in 5400 lethal in 18 min.; and 1 part in 7500 non-lethal) are shown on the right-hand ordinate in bold type.

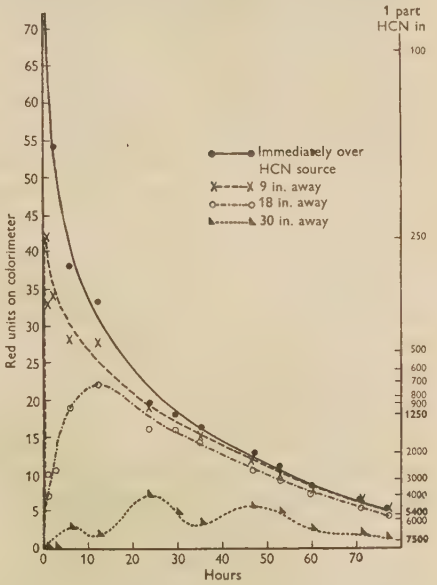

Fig. 3. Persistence of hydrogen cyanide concentrations with 1 oz. (28·3 g.) of Cyanogas under summer conditions. Curves drawn freehand through the points shown. One point has been omitted. The peak of the curve immediately over the hydrogen cyanide source came at ½ hr. at a concentration of 1 part in 57. Critical levels are shown on the right-hand ordinate in bold type.

Again, samples were taken from point 5 with consistently negative results. The amount of diffusion of hydrogen cyanide down the burrow was in general much the same as with Cymag except that point 4 showed a rather higher concentration and maintained it for a longer time.

The curves for points 1, 2 and 3 are the most interesting of the results. Within the first half an hour the concentration jumped to a very high level (not precisely determined, but greater than 1 part in 50 or 24 mg./l.). This peak is much more

marked immediately over the source of the gas than at point 2 (9 in. away), while at point 3 the shape of the curve is not very different from that for Cymag.

Even more marked is the quick falling away of this very high concentration. Point 2 indeed shows it after only 1 hr., while at point 1 the shape of the curve is the same, although the fall to a 1/1250 level is not so sudden owing to the much higher peak that has been reached. By the end of 24 hr. the curves for points 1, 2 and 3 have all finished their quickest rate of fall and have come to the region of about 1 part in 1000 (1·2 mg./l.). They then approximate and up to about 80 hr. they decline gradually much in the same way as Cymag does from about 55 hr. onwards. The greatest difference between the two powders is in the speed with which the concentrations fall below the level of 1 part in 1250 (1 mg./l.): at point 1 this occurs after 34 hr., compared with 48 hr. in the case of Cymag.

Exp. C (Fig. 4). This and the following experiment repeat Exps. A and B, but with the dose reduced to 0·5 oz., to determine whether this economy could be made without reducing the efficiency of operations too much. The conditions of temperature and humidity were almost identical in this and in Exp. D with the exception that the range of temperatures dropped by about 2° C. in the latter. In comparison with Exps. A and B, the relative humidity reached exactly the same level (86%), but was somewhat slower in building up; the temperature was about 3° C. higher, which may have slightly accelerated the release of the gas.

The curve for the 0·5 oz. dose of Cymag (Fig. 4) falls away more quickly than with the 1 oz. dose and lacks the blunt peak that was so characteristic of the latter. This suggests that the equilibrium effect is not nearly so marked and that the smaller the dose the more nearly will the curve approximate to the Cyanogas type. The concentration at the source (point 1) has already fallen to 1 part in 1250 (1 mg./l.) at the end of 20 hr., and it continues to fall steeply (the flattening-out noted in Exp. A is missing here) until it reaches a level of 1 part in 5400 (0·22 mg./l.) in 37 hr. (compared with 84 hr. in Exp. A).

There is also less diffusion down the burrow than with the 1 oz. dose, since only a trace is recorded at point 4 (30 in. from the source) throughout the experiment.

Exp. D (Fig. 5). The curve with 0·5 oz. of Cyanogas is a replica on a smaller scale of the 1 oz. test. There is the same high initial peak with a quick falling-away and then a slight flattening-out. From the point of view of practical gassing technique, however, the efficiency is much impaired: the concentration at point 1 has fallen below the level of 1 part in 1250 (1 mg./l.) in 11 hr. only and 1 part in 5400 (0·22 mg./l.) is reached in 40 hr. Diffusion is so slight that point 4 gave consistently negative results throughout and so does not appear in the figure.

A test with Cyanogas G (coarse ground) showed that it was quite unsatisfactory for rabbit gassing, since the surface layer of powder, when exposed to the air, formed a hard crust, preventing the powder inside from reacting with the moisture in the air.

It will be useful to summarize the results of Exps. A–D, carried out under summer conditions, before describing those done in the winter.

(1) The smaller dose of either material is inadequate for optimum results, since a satisfactory concentration (1 part in 1250) is not maintained for long enough to cover the first night.

(2) The larger dose gives satisfactory results with either material, but the curve for Cymag shows a buffering effect, prolonging the peak. Thus a concentration above 1 part in 1250 is maintained for 48 hr. with Cymag, but only for 33 hr. with

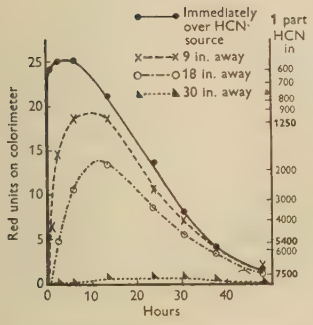

Fig. 4. Persistence of hydrogen cyanide concentrations with ½ oz. (14·15 g.) of Cymag under summer conditions. Curves drawn freehand through the points shown. Critical concentrations are shown on the right-hand ordinate in bold type.

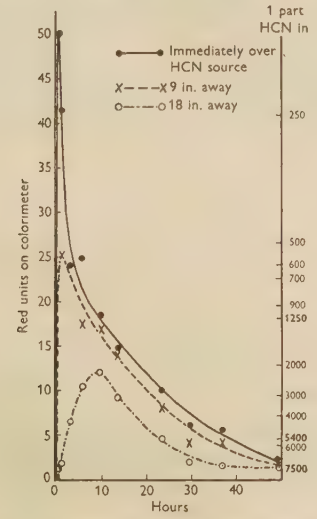

Fig. 5. Persistence of hydrogen cyanide concentrations with ½ oz. (14·15 g.) of Cyanogas under summer conditions. Curves drawn freehand through the points shown. Point 4 (30 in. away from the hydrogen cyanide source) was consistently negative throughout the experiment. Critical concentrations are shown on the right-hand ordinate in bold type.

Cyanogas. In the latter case the second night after the gassing would not be adequately covered, unless gassing was done in the late afternoon.

(3) A medium concentration (1 part in 5400) persists for the same length of time, i.e. about 80 hr., with either material. This concentration would probably kill some rabbits (see p. 341).

(4) The very large initial peak with Cyanogas makes it the better material for using with a foot pump, but not for 'spooning'.

(5) If the powder is placed in as compact a heap as possible, this does not prevent the building up of a high concentration within ½ hr.

(6) Diffusion down the burrow is slight, since 42 in. away from the source of the gas no trace was recorded throughout the experiments. Thus the maximum concentration is built up just inside the entrance to the burrow.

(7) To ensure that a high concentration with 1 oz. of Cyanogas lasts over the second night after gassing, it will be necessary to gas late in the day. With Cymag this point does not apply.

Winter experiments

These were carried out in exactly the same way as Exps. A–D and were confined to testing the 1 oz. doses of both materials. The relative humidity was higher than in the summer (90–92% compared with 86%), but this would have no effect on the rate of evolution of the gas. The difference is, therefore, entirely accountable to the changed temperature, which was consistently 10° C. below summer conditions.

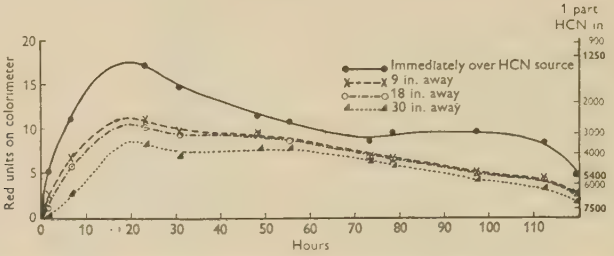

Fig. 6. Persistence of hydrogen cyanide concentrations with 1 oz. (28·3 g.) of Cymag under winter conditions. Curves drawn freehand through the points shown. Critical concentrations are shown on the right-hand ordinate in bold type.

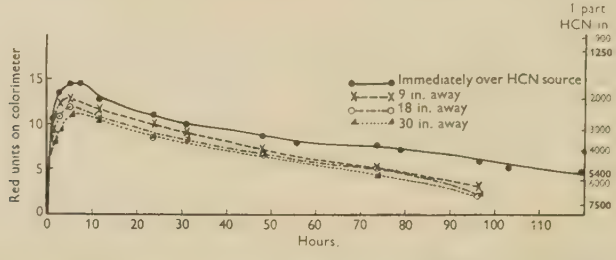

Fig. 7. Persistence of hydrogen cyanide concentrations with 1 oz. (28·3 g.) of Cyanogas under winter conditions. Curves drawn freehand through the points shown. Critical concentrations are shown on the right-hand ordinate in bold type.

Exp. E (Fig. 6). This was done with 1 oz. of Cymag, and the curve shows considerable modification in the following details.

(*a*) The typical flat-topped curve remains, but it has been squashed down greatly, so that concentrations never reach so high a level as they did in the summer. There is considerable delay (15 hr.) in reaching a concentration of 1 part in 1250.

(*b*) The persistence of the gas is notably increased. At point 1 the concentration

has fallen in summer to a level of 1 part in 5400 by 80 hr., whereas in winter this does not occur until 127 hr., or over 5 days, and as long as 112 hr. a reasonable medium concentration (1 part in 3400 or 0·35 mg./l.) remains.

(*c*) The rate of absorption of the gas by the walls of the burrow is also slowed down. This factor is partly responsible for the prolongation of the flat top to the curve, but its effect is as clearly seen in the greater rate of diffusion along the burrow. If the curve for point 4 is compared with that given in Fig. 2, it is seen to reach a higher concentration (1 part in 3500) and persists at a medium strength for much longer: in winter it has declined to 1 part in 5400 at the end of 96 hr., whereas in summer it barely reaches this level at all. The general effect is clearly shown in the smaller amount of disparity between the curves for the four points. This means that a greater percentage of the total length of burrow will be lethal to rabbits in winter than in summer.

Exp. F (Fig. 7). In this case the typical Cyanogas curve is discernible, but again there are differences.

(*a*) The peak concentration is remarkably reduced compared with the summer (1 part in 1400, or 0·73 mg./l. instead of 1 part in 50 or 24 mg./l. at point 1). This is lower than the peak reached by Cymag.

(*b*) The concentration rises to this peak in a reasonably quick time (5 hr.) in spite of the lower temperature. In this way Cyanogas is superior to Cymag.

(*c*) The fall of the concentration from this peak is remarkably prolonged and steady, taking 120 hr. to reach 1 part in 5400. In this way the results are not unlike those with Cymag, though the middle part of the curve is consistently a little higher with the latter material.

(*d*) Greater speed of diffusion is marked here also. At the beginning of the experiment the curves for all four points progress almost simultaneously for the first hour. Point 4, which in summer only rose above 1 part in 5400 after the experiment had been going for 15 hr. and declined below it again at 55 hr., rises above that level at once and does not fall to it again until 72 hr. Points 2 and 3 follow this very closely.

In winter, therefore, lethal concentrations are maintained in the burrows for much longer than in summer, which is advantageous because rabbits will lie up for much longer at this time of year. Cyanogas, however, never reaches a 'killing' concentration, so that Cymag holds more promise for winter work. The only drawback to the latter is its slowness in getting off the mark, so that it should either be used early in the day, or should be rather more scattered when placed in the burrows.

Toxicity of hydrogen cyanide to rabbits

Toxicity curves are usually constructed to show the time at various concentrations needed to kill 50% of the animals exposed. Such a curve for rabbits has been published by Barcroft (1931), and is reproduced in a modified form in Fig. 8.

Two preliminary points need discussion. In the first place the concentrations given by Barcroft are 'nominal', which means that a stated concentration was introduced into the chamber at the beginning of each experiment and assumed to have remained at this level throughout. In fact in one test that was made a concentration of 1 part in 5250 (0·23 mg./l.) lost 22% by absorption on the walls of the chamber and by intake into the animals at the end of 38 min. This means that the right-hand end of this curve should be lowered.

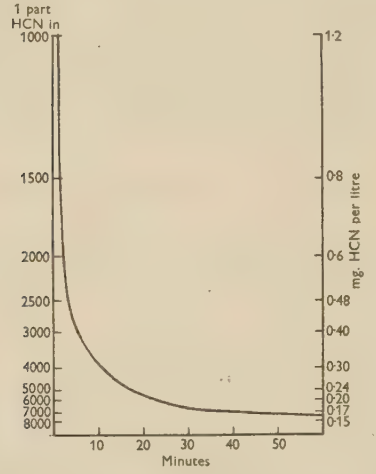

Fig. 8. Toxicity curve (after Barcroft) showing the length of exposure to each concentration of hydrogen cyanide necessary to kill 50% of rabbits exposed.

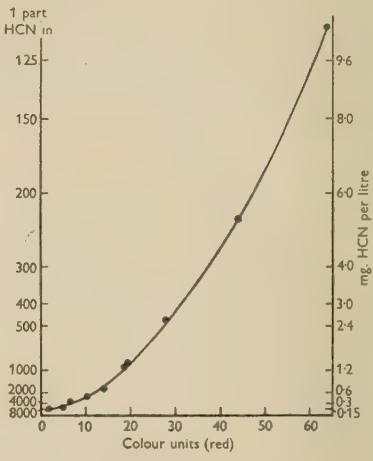

Fig. 9. Relation between red colour units on Lovibond-Schofield Tintometer and concentrations of hydrogen cyanide. The points, through which the freehand curve has been drawn, were determined from standard solutions containing amounts of hydrogen cyanide determined by titration. One further point for a concentration of 1 part in 57 (21 mg./l.), which gave a reading of 80 red colour units, has been omitted.

Furthermore, a distinction should be made between the time of collapse and death. Data for the goat give the time ratio, collapse : death, as 1 : 3. Barcroft (personal communication) affirmed that this also applies to other animals, and therefore the time of collapse will be more important, since, unless the concentration were to drop suddenly, there would be no chance of the rabbit's recovery except at very low levels. In fact, this last point is not of practical importance because this level is only reached at about 1 part in 10,000 (0·12 mg./l.).

These two considerations mean that, if the curve errs at all, it will be in the direction of underestimating the toxicity of hydrogen cyanide to rabbits.

The critical points on this curve, which are shown in Figs. 2–7 in bold type to facilitate interpretation of the experimental results, are the following: (*a*) 1 part in 1250 (1 mg./l.) which is toxic to 50% of the rabbits exposed in 1 min.; (*b*) 1 part in 5400* (0·22 mg./l.) which is toxic in about 18 min.; and (*c*) 1 part in 7500 (0·16 mg./l.) which one can roughly take as the limit of toxicity.

From a practical point of view it is rather unsatisfactory to take points indicating a 50% chance of recovery, and information giving a 90% kill or thereabouts is desirable. Barcroft's data are not sufficient to reconstruct the curve, but some indication of the amount of modification is given in Weedon, Hartzell & Setterstrom (1940). These authors give toxicity figures for 87·5% success, but they should be applied tentatively since they refer to rats and not to rabbits. Barcroft's work shows that there is a slight difference between rabbits and rats (the latter are slightly less susceptible to high and more susceptible to low concentrations).

The new data show that an increase from 50 to 87·5% kill is attained at 1 min. by an additional time of exposure of 0·75 min., and at 18 min. by an addition of 3 min. These differences should be borne in mind, but the 50% levels must still be regarded as the basic data.

Discussion

Any consideration of the bearing of the above data on practical gassing technique must also take into account the behaviour of the rabbit. At an undisturbed warren during the summer feeding activity commences as a rule some hours before sunset. Feeding in the middle of the day is not usual, though it may occur under exceptional weather conditions. Observations made at a rabbit warren, in which a proportion of the rabbits were marked and the total population known approximately (Southern, 1940), showed that during the evening usually about one-third of the total was feeding above ground. Furthermore, evidence based on the proportion of rabbits trapped at different times of the day and night with 'soft' faecal pellets in the stomach, shows a clear feeding rhythm with two peaks (Southern, 1942). These soft, lobular pellets are taken direct from the anus and reingested, and their presence in the stomach denotes that the animal concerned was at a resting phase of its daily rhythm.† These data show that during March a very high percentage of rabbits caught between 6 a.m. and 4 p.m. were not feeding on fresh food. After this the proportion with fresh food in the stomach increased to a peak (95%) between 7 and 11 p.m., fell again between the latter time and 2 a.m., then rose to a peak of 100% between 2 and 6 a.m. It is evident from this that most of the rabbits in a warren will be out feeding at and just after dusk and again during the middle of the night. Other things being equal, it should be possible to ensure that practically all the population of a warren is gassed during one night provided that an adequate

* This concentration is chosen rather arbitrarily, but it is probably near the lower limit for practical effectiveness.

† This does not necessarily mean that it will not come above ground, but that it is far less likely to do so, especially when disturbed, since the principal motive is in abeyance.

concentration is present during the evening. If 1 oz. of Cymag is placed within the entrance of each burrow at any time during the day and well sealed in, there should theoretically be a concentration of well over 1 part in 1250 (1 mg./l.) by the time the rabbits start to come out and feed.

There are, however, two difficulties to be encountered. In the first place any disturbance to the environment of the rabbit produces a marked shyness in their behaviour. Blocking in all the burrow mouths cannot be done without disturbing the occupants and their reaction is to lie up in the lower galleries until hunger overcomes their nervousness. The time taken for this to happen varies according to the season and to the amount of disturbance they have undergone. In winter they have been known to stay underground for some days and in the later stages of an intensive trapping campaign they become notoriously shy. No positive information exists on the length of time they will lie up in these circumstances, but trappers mention that 3 days is not unusual, and sometimes the period is even longer.

Some experiments were undertaken by the writer during summer and early autumn to test the amount of shyness occasioned by simply filling in all the entrances to a warren. The results are given in Table 1.

TABLE 1. *Records of hole opening at blocked rabbit warrens*

Warren	Month	Total no. of holes opened 3 weeks after blocking	No. of holes (% of total) freshly opened on night			
			1	2	3	4
A	August	40	30	0	0	0
B	October	7	43	0	0	0
C	October	13	45	0	—	—
D	October	9	55	12	—	—
E	October	27	12	21	0	—
F	October	19	47	11	27	—
G	July	48	—	—	1	28

A dash indicates that no observation was made on that day. The total number of holes blocked in is of no importance because many were old and unused. Figures for nights 1–4 are, therefore, expressed as percentages of the total opened 3 weeks after blocking.

The figures for the first 4 days after the initial blocking-in are given because it seems reasonable to suppose that all rabbits would have found a way out by then, and that further increase in the number of holes opened would be mere elaboration.

The results show considerable variability. At warrens A, B and C there is no doubt that complete activity was resumed on the first night and therefore a lethal concentration of gas need only have been maintained for 24 hr. But at warrens D and E it is probable that some rabbits did not become active until the second night, while at F there was still further hole opening on the third night. Finally at warren G the rabbits remained almost completely inactive until the fourth night. This last record is probably unusual for the time of year, but it illustrates the variations in activity which plans for gassing should take into account. It is probable that even wider

variation might have been encountered during the winter, but no observations were made then.

We do not know how long it will take a rabbit to scratch its way through the blocked entrance to a burrow: in the absence of precise data it is not unreasonable to suppose that a concentration causing death in 1 min. (and therefore collapse in about 20 sec.) would be almost certainly fatal, while a concentration causing death in 18 min. (and collapse in 6 min.) would stand a fair chance of being fatal. Figs. 2 and 3 show that 1 oz. of Cymag will provide a 'sure' concentration (lethal in 1 min.) for the first 2 nights. Cyanogas will not last for so long at this level unless applied late in the day, and therefore would only have been at its most efficient for three of the warrens in Table 1, whereas Cymag should have covered all but C and G. This, of course, is not to say that subsequent deaths would not have been caused by concentrations between the 1 and 18 min. lethality lines, and, as far as this goes, Cymag has only a slight advantage over Cyanogas.

These considerations only apply to summer. If we turn to Figs. 6 and 7 we see that in winter a medium concentration (lethal in 18 min.) is maintained for a very long time (about 5 days), which should be an automatic adjustment to the longer time for which rabbits are said to lie up in cold weather and during winter trapping campaigns. On the other hand, a 'sure' concentration is maintained for only a short time with Cymag and is not reached at all with Cyanogas. It is difficult to say, without further data on the time for which rabbits will expose themselves to the gas, what success these winter curves predict. Middleton's experiments (unpublished) in gassing and digging out warrens in the winter show that the 1 oz. dose produces very satisfactory results. Of thirteen warrens with a total of 331 holes, most of which were left for 48 hr. before being dug out, only three holes were found opened; seventy-seven dead rabbits were recovered and no live ones at all.

These results suggest that the concentrations which kill in from 1 to 18 min. are sufficiently successful. However, where economy is not the primary object, it would doubtless be of advantage to increase the dose of powder during the winter.

The second difficulty that needs considering is whether the building up of a concentration immediately after treatment is quick enough to anticipate the recommencement of activity under all circumstances. In summer the level of 1 part in 1250 is reached within half an hour, and probably very much earlier. There is little doubt that Cyanogas, which has built up a concentration of more than 1 part in 50 by the end of half an hour, will reach 1 part in 1250 in a very few minutes. Cymag, reaching only 1 part in 960 (1·25 mg./l.), is slower. The only possible source of failure could come from very small rabbits, which are not so shy as the older ones, and may start to scratch out within a few minutes of the warren being blocked in. It seems probable that during this time of year extra care in blocking the holes is necessary to make quite sure that scratching out is impossible before a lethal concentration is reached. As an extra precaution Cyanogas may be used, if it is available.

In winter this problem presents rather a different picture. On the one hand, there are no small rabbits to start immediate scratching out, and the adults are more shy than at any other time of year; on the other hand, the lower temperatures may retard the building up of a peak concentration for some 10–15 hr. In these circumstances it is probably better to gas warrens early in the day. Quicker evolution of gas can be obtained by exposing more surface area of the powder, but this would certainly mean a lowering of the concentration after the first or second day. Further research is needed on this point to discover whether more efficient conditions can be provided by an increased dose together with more scattering of the powder.

This work was done under a wartime grant to the Bureau of Animal Population from the Agricultural Research Council. The help of Dr R. E. Slade and of Dr F. P. Coyne, Imperial Chemical Industries Ltd., in supplying data on the rate of evolution of hydrogen cyanide from Cymag and Cyanogas under different conditions in the laboratory is gratefully acknowledged. The late Sir Joseph Barcroft also very kindly advised on the estimation of toxicity. Some other acknowledgements are made in the Appendix on the method used for estimating hydrogen cyanide concentrations.

APPENDIX

A rapid method of making estimations of hydrogen cyanide concentrations in the field

Previously described methods for measuring the concentrations of HCN gas have mostly been designed for checking the danger to human beings. Tests such as the benzidine-copper acetate, congo red, methyl orange-mercuric chloride (Anon., 1938; Page & Gloyns, 1936; O'Donnell, Mundt, Knudsen & Delano, 1940; Sherrard, 1928) test for ranges, whose upper limit is about 1 part in 10,000 (0·12 mg./l.) and generally assume an unlimited supply of the atmosphere it is desired to test. Furthermore, most of them employ a filter-paper soaked in the indicator solution. Arranging to have a supply of these in the field is not easy.

The alkaline picrate test (Guignard, 1907; Adriano & Ynalvez, 1932) was finally adopted as a basis for the method used. For each field test 625 ml. of air-HCN mixture were drawn from the experimental burrow through one of the sampling tubes by means of a hand-exhausting pump (capacity 125 ml.). The HCN was absorbed by passing through 26 ml. of indicator solution prepared by diluting 3 ml. of Guignard's stock solution* with 23 ml. of distilled water. A rapid change in colour from yellow to red takes place and the depth of the red colour (indicating the concentration of HCN) was measured with a Lovibond-Schofield Tintometer. Standard curves were constructed from KCN solutions of known concentrations.

The test is based on the colour change due to the formation of isopurpuric acid, when HCN is absorbed into a solution of picric acid and sodium carbonate. The D.S.I.R. Leaflet mentions the test, but does not recommend it because it is not specific, reaction also taking place with hydrogen sulphide, acetone, aldehydes, etc. This drawback, of course, is unimportant for the present purpose.

The hand-exhausting pump (capacity 125 ml.) used in the field tests was the same as that supplied by Messrs Griffiths and Tatlock for measuring traces of HCN in industrial premises

* Made up in the proportions of 1 part by weight sodium carbonate (cryst.) to 10 parts 1 % aqueous solution of picric acid.

after fumigation. A series of small absorption tubes was prepared so that a number of samples could be drawn in succession. A rubber bung bored to take two glass tubes, several lengths of rubber tubing for connexions and a wire holder fixed to the exhausting pump to keep the absorption tube steady were also necessary. With this apparatus samples were withdrawn from each point along the artificial burrow in turn.

For each test five strokes of the pump were made, thus removing 625 ml. of air-HCN mixture from the burrow. Supposing that the concentration of HCN were 1 part in 1000 by volume (1·2 mg./l.) the amount of HCN in 625 ml. would be 0·75 mg. Thus a solution of KCN made up with 0·75 mg. HCN in a stated volume of water (determined for convenience of colour measurement as 23 ml. (see below)) would give a colour reaction characteristic for 1 part of HCN in 1000 by volume.

For preparing a series of standard colours, from which to construct curves, Dr H. Irving of the Inorganic Chemistry Department, Oxford, kindly supplied solutions of 0·04837 N, 0·00430 N and 0·001075 N-KCN determined by titration.

The simplest procedure was to dilute these solutions in simple ratios ($\times 2$, $\times 4$, etc.) and construct a curve from the points thus determined (Fig. 9). In this way the 0·001075 N solution would give a colour reaction equivalent to an HCN concentration of 1 part in 1117*; diluted $\times 2$ it would represent 1 part in 2234, etc.

Next a test was made to determine the amount of stock alkaline picrate solution to be added for making each colour standard (and, of course, for treating each sample in the field work). It was found that 2 ml. of the stock added to a solution containing 1·16 mg. HCN (the equivalent of sampling a burrow concentration of 1 part in 647 by volume) was in excess of requirements (the addition of further alkaline picrate produced no deeper colour). Thus it was thought safe to add 3 ml. of alkaline picrate as a standard procedure, which would cover the occasional measurement of even higher concentrations. Each colour standard solution, therefore, was made up to a total of 26 ml. and each absorption tube used in the actual experiments contained the same total amount (3 ml. stock alkaline picrate and 23 ml. distilled water).

Estimation of the standard solutions and of the samples was carried out in the Lovibond-Schofield Tintometer. The curve constructed by measuring the standard colours is shown in Fig. 9.

To establish that the absorption of HCN was completed by passage through a single tube of alkaline picrate solution a second absorption tube containing an equal amount of indicator solution was inserted in series, so that the air-HCN mixture from the burrow was drawn through two lots of indicator successively. Any colour change in the second indicator would betray incomplete absorption in the first. No such change was observed while the mixture was drawn through at a normal rate (1·5 ml./sec.). It was necessary to race the mixture through (>5 ml./sec.) for any HCN to pass over into the second tube.

Simple tests were also devised to check whether any of the HCN in the air drawn from the burrow was absorbed on the inside of the connecting tubes before reaching the indicator solution, and whether any HCN remained in solution on the moist walls of the burrow long enough after an experiment was done to interfere with the next test. Both factors were negligible.

The following acknowledgements are gratefully made: to Sir Robert Robinson, Dyson Perrins Laboratory, for advice; to Dr S. G. Plant, Dyson Perrins Laboratory, for advice and

* N solution contains 27 g. HCN/l.
0·001075 N solution contains 0·0292 g./l. or 29·2 mg./l. = 0·06716 mg./23 ml.

Since 0·75 mg./23 ml. is equivalent to a concentration of 0·001 HCN (see above), 0·6716 mg./23 ml. is equivalent to a concentration of $\frac{0{\cdot}001 \times 0{\cdot}6716}{0{\cdot}75} = 0{\cdot}0008954 = 1/1117$.

the loan of apparatus; and finally to Dr H. Irving, Inorganic Chemistry Department, for continual help and discussion, especially in preparing this account, and for providing the standard KCN solutions.

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STATISTICAL ASPECTS OF THE INDEPENDENT JOINT ACTION OF POISONS, PARTICULARLY INSECTICIDES

I. THE TOXICITY OF A MIXTURE OF POISONS

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(With 5 Text-figures)

Bliss's (1939) statistical treatment of the hypothesis of the independent joint action of poisons is extended and revised. The possibility of negative correlation in resistance is considered, and the bivariate normal correlation coefficient is used to measure correlation in resistance. There are interesting characteristics of the dosage-mortality curve for a mixture of poisons the resistances to which are log-normally distributed: these are described. The question of whether a mixture of poisons is more toxic if they act similarly or independently is discussed.

Introduction

Bliss (1939) defined three possible physiological types of joint action between poisons—independent, similar, and synergistic, and considered antagonism as negative synergism. Trevan (1927) had foreshadowed Bliss's concepts of independent and similar action. Only physiological investigation of the mode of action of poisons applied separately and jointly can finally determine the type of action between them; but since, as Bliss showed, the relation between the toxicity of a mixture of poisons and the toxicities of its separate constituents will differ according to their mode of joint action, statistical examination of dosage-mortality data may strongly indicate how poisons act together.

Most of the work on the joint action of insecticides has been statistical, and not physiological; and most, not on independent action, but on similar action, synergism, and antagonism. Finney (1942, 1947) improved Bliss's mathematical treatment of the hypotheses of similar action and synergism. Sets of dosage-mortality data analysed by Finney (1942) and Swisher (1943) were consistent with the hypothesis of similar action. Bliss (1939) found statistical evidence for two examples of synergism. Activation, a limiting case of synergism in which one 'poison' is non-toxic in the range of dosage used, is known to occur (see, for example, Haller, Laforge & Sullivan, 1942). As Swisher (1943) pointed out, the term 'synergism' has been loosely used, and many of the other examples of supposed synergism may not be synergism as defined by Bliss. Shepard & Lindgren (1934), Jefferson (1943), and Parkin (1946) recorded antagonism between certain fumigants.

The statistical evidence that insecticides may act independently is less satisfactory. Bliss thought that if two poisons act independently, the dosage-mortality curve for a mixture of the two is likely to change relatively suddenly in slope. He pointed out that a dosage-mortality curve given by Murray (1938) for pyrethrins against houseflies showed this feature, and suggested that the pyrethrin I and pyrethrin II might therefore have acted independently. He considered that the discontinuous dosage-mortality curves given by some poisons when used alone could be explained on the assumption that a poison may act on two independent systems in the organism, producing curves similar to those for two poisons acting independently. Never, it appears, has the toxicity of a mixture of insecticides been satisfactorily predicted from the toxicities of its constituents on the assumption that they acted independently.

In examining dosage-mortality data to see whether they were consistent with the hypothesis of independent action, we found Bliss's statistical treatment of the hypothesis inadequate, and have therefore extended and revised it. We have considered the independent action of two poisons only, but our equations could easily be generalized to three or more. Death is the only response, and insects the only organisms, mentioned in this paper, but the mathematical concepts could apply equally to other all-or-none responses and to other types of organism.

In a second paper we shall describe methods for testing dosage-mortality data for agreement with the independent action hypothesis; apply these methods to the joint action of pyrethrins, D.D.T., and benzene hexachloride, taken in pairs; and discuss how synergism could arise from independent action.

The primary purpose of the present paper and its sequel is to provide methods for testing statistically whether given poisons may act independently or not.

PREDICTION OF THE TOXICITY OF A MIXTURE OF POISONS FROM THE TOXICITIES OF ITS SEPARATE CONSTITUENTS

According to Bliss's (1939) definition, when two poisons act independently they cause different physiological systems in the insect to fail, so that the action of one poison upon an individual insect neither augments nor interferes with that of the other. If two poisons, A and B, act simultaneously but independently upon an insect, it will die if it receives a lethal dose of A, or a lethal dose of B, or lethal doses of both A and B: it will survive only if it receives sublethal doses of both A and B. If it receives lethal doses of both poisons, which poison kills the insect may be in doubt, but that it dies is the important fact here.

The toxicity of a mixture containing A and B is not uniquely determined by the toxicities of A and B applied separately. In order to predict the proportion of insects killed by a mixture of A and B, it is necessary to know, not only the proportions that would be killed if A and B were applied separately, but also to what extent, if at all, resistance* to A is correlated with resistance to B within the group

* Measured by the individual minimum lethal dose.

of insects treated. If resistance to A is completely and positively correlated with resistance to B, an individual insect very resistant to A in comparison with its fellows will be very resistant to B also. If the correlation is complete and negative, an insect very resistant to A will be correspondingly very susceptible to B. If there is no correlation, the resistance of an insect to A will be unrelated to its resistance to B. Resistance to A may, of course, be incompletely correlated with resistance to B, in which case if an insect is very resistant to A, there will be a probability, larger or smaller according to the degree of correlation, that it will be very resistant or very susceptible to B.

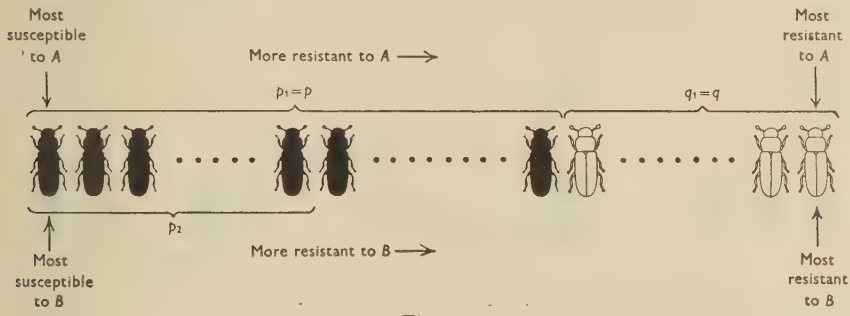

Fig. 1

Firstly, suppose that resistance to A and resistance to B are completely and positively correlated. Let given doses of A and B applied separately kill proportions p_1 and p_2 respectively of the insects treated, and let the same doses applied together kill a proportion p. Let the corresponding proportions surviving be $q_1=1-p_1$, $q_2=1-p_2$, and $q=1-p$. Let $p_1 \geqslant p_2$. Fig 1 illustrates the relation between p_1, p_2, and p. In the figure, a group of insects treated with A and B together is represented diagrammatically, the insects being arranged in order of their resistance to A, with the insect most susceptible to A on the left and that most resistant to A on the right. As resistance to A and to B are completely and positively correlated, the insects will have been similarly arranged in order of their resistance to B. The insects that die are shown in solid black and those that survive in outline. The brackets are marked with the proportions of the group that they enclose, and those marked p_1 and p_2 enclose respectively the insects receiving lethal doses of A and those receiving lethal doses of B. Both these brackets extend from the left-hand end of the line of insects because the insects on the left are those most susceptible to both A and B. As $p_1 \geqslant p_2$, the bracket marked p_1 is shown as longer than that marked p_2: no insect outside the former bracket will receive lethal doses of either A or B, so that

$$p=p_1 \qquad (p_1 \geqslant p_2). \tag{1}$$

It can similarly be shown that

$$p=p_2 \qquad (p_2 \geqslant p_1). \tag{2}$$

Secondly, suppose that resistance to A and resistance to B are uncorrelated. A proportion p_1 will receive lethal doses of A, and be killed. Since resistance to A and to B are uncorrelated, the remainder, the proportion q_1, may be considered, so far as its resistance to B is concerned, as a random sample of the group of insects treated. Of this proportion q_1 a proportion p_2 will therefore be killed, i.e. a proportion $p_2q_1=p_2-p_1p_2$ of the whole group treated. Hence the total proportion killed will be

$$p=p_1+p_2-p_1p_2. \tag{3}$$

When the resistances are uncorrelated, (3) is true whatever the magnitudes of p_1 and p_2.

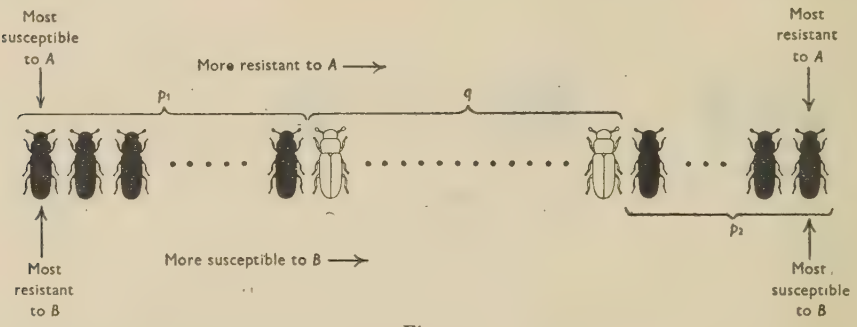

Fig. 2

Thirdly, suppose that resistance to A and resistance to B are completely and negatively correlated. Let $p_1+p_2\leqslant 1$. Fig. 2 illustrates the relation between p_1, p_2, and p. The brackets marked p_1 and p_2 now extend from opposite ends of the line of insects so that

$$p=p_1+p_2 \quad (p_1+p_2\leqslant 1). \tag{4}$$

If $p_1+p_2\geqslant 1$, the brackets marked p_1 and p_2 extend inwards until they meet or overlap, so that

$$p=1 \qquad (p_1+p_2\geqslant 1). \tag{5}$$

Equations (1)–(5) can be derived in a more formal way, the value of which will become apparent later. If α and β are respectively the classes of insects receiving lethal doses of A and B, and $\bar{\alpha}$ and $\bar{\beta}$ are respectively the classes of insects receiving sublethal doses of A and B, when a group of insects is treated with A and B together the group can be divided into four classes, $\alpha\beta$, $\bar{\alpha}\beta$, $\alpha\bar{\beta}$, and $\bar{\alpha}\bar{\beta}$. The proportions of the group falling within these four classes can be shown in contingency tables. For example, when there is complete positive correlation in susceptibility and $p_1\geqslant p_2$, the table is as follows:

	α	$\bar{\alpha}$
β	p_2	0
$\bar{\beta}$	p_1-p_2	q_1

Complete positive correlation
$p_1\geqslant p_2$

This table corresponds to equation (1). It can be seen from Fig. 1 that the class $\alpha\beta$, containing insects receiving lethal loses of both A and B, comprises a proportion p_2, for the insects enclosed in the bracket marked p_2 are also enclosed in that marked p_1. Similarly, $\alpha\bar{\beta}$ contains the insects inside the bracket marked p_1, but outside that marked p_2. No insect receives a lethal dose of B and a sublethal dose of A, i.e. the proportion in $\bar{\alpha}\beta$ is zero. All the insects in the classes $\alpha\beta$, $\bar{\alpha}\beta$ and $\alpha\bar{\beta}$ will die, so that p is the sum of all the entries in the table save the lower right-hand one.

The tables corresponding to equations (2)–(5) can be worked out in the same way and are as follows:

	α	$\bar{\alpha}$	
β	p_1	p_2-p_1	Complete positive correlation $p_2 \geqslant p_1$
$\bar{\beta}$	0	q_2	

	α	$\bar{\alpha}$	
β	p_1p_2	q_1p_2	No correlation
$\bar{\beta}$	p_1q_2	q_1q_2	

	α	$\bar{\alpha}$	
β	0	p_2	Complete negative correlation $p_1+p_2 \leqslant 1$
$\bar{\beta}$	p_1	q_1-p_2	

	α	$\bar{\alpha}$	
β	p_1-q_2	q_1	Complete negative correlation $p_1+p_2 \geqslant 1$
$\bar{\beta}$	q_2	0	

Generalization of equations (1)–(5)

Bliss (1939) considered only the possibilities of positive correlation or absence of correlation in resistance, and derived, in effect, equations (1), (2), and (3). He did not consider the possibility of negative correlation, which has been mentioned by Wadley (1945). Bliss surmised that the degree of correlation could be measured by a correlation coefficient r, and that p could be equated to functions of p_1, p_2, and r. When $p_1 > p_2$, he suggested that (in our notation)

$$p = p_1 + p_2(1-p_1)(1-r), \tag{6}$$

which is the simplest polynomial in r that reduces to (1) when $r = 1$ and to (3) when $r = 0$. By substituting the value of r in (6), he thought that the relation between p_1, p_2, and p might be obtained when the correlation in resistance was positive and incomplete, i.e. $0 < r < 1$. As might be expected, since Bliss did not consider

the possibility of negative correlation, (6) does not reduce to (4) or (5) when $r=-1$, so that (6) is not a generalization of (1)–(5).

Equations (1)–(5) could be generalized by equating p to quadratics in r. We consider this unsatisfactory, however, because p must then be equated to different quadratics according as

(*a*) $q_2 \geqslant p_1 \geqslant p_2$ $(p_2 \leqslant \frac{1}{2})$, (*c*) $p_1 \geqslant p_2 \geqslant q_1$ $(p_1 \geqslant \frac{1}{2})$,

(*b*) $q_1 \geqslant p_2 \geqslant p_1$ $(p_1 \leqslant \frac{1}{2})$, (*d*) $p_2 \geqslant p_1 \geqslant q_2$ $(p_2 \geqslant \frac{1}{2})$.

For example, the simplest generalization of (1), (3), and (4), which correspond to (*a*), is

$$p = p_1 + p_2 - p_1 p_2 - \tfrac{1}{2} p_2 r + \tfrac{1}{2}(2p_1 p_2 - p_2) r^2. \tag{7}$$

Unless p_1 and p_2 are restricted as in (*a*), (*b*), (*c*), or (*d*), to relate p_1, p_2, and p at different doses will require more than one quadratic (in general three). r cannot have the same meaning in each, and cannot, therefore, be uniquely defined.

Use of the bivariate normal correlation coefficient. What is evidently required is a single function expressing p in terms of p_1, p_2, and a parameter ρ $(-1 \leqslant \rho \leqslant 1)$, measuring the correlation in resistances to A and B. This function must be such that equations (1) and (2), (3), and (4) and (5), appear when ρ is put equal to $+1$, 0, or -1 respectively. A number of functions containing double integrals satisfy this condition. In particular, the integrated bivariate normal distribution, in standard form, with zero means, unit variances, and correlation coefficient ρ, effectively generalizes (1)–(5) when the limits of integration are suitable functions of p_1 and p_2, i.e. the corresponding normal equivalent deviations (N.E.D.).*

Let x_1, x_2, and x, then, be the N.E.D.'s corresponding to p_1, p_2, and p respectively, and let

$$F(u) = \int_{-\infty}^{u} f(t)\, dt \quad \text{and} \quad f(t) = \exp[-\tfrac{1}{2}t^2]/\sqrt{(2\pi)}, \tag{8}$$

so that $p_1 = F(x_1)$; $q_1 = F(-x_1)$; $p_2 = F(x_2)$; $q_2 = F(-x_2)$; $p = F(x)$; $q = F(-x)$. q can be more simply expressed than p, and is given by

$$q = \int_{-\infty}^{-x_1}\int_{-\infty}^{-x_2} \frac{1}{2\pi\sqrt{(1-\rho^2)}} \exp\left[-\frac{1}{2(1-\rho^2)}(u^2 - 2\rho uv + v^2)\right] du\, dv, \tag{9}$$

where $-1 \leqslant \rho \leqslant +1$.

Equation (9) is very general. In using it to define the relation between q, p_1, p_2, and ρ, the resistances to A and to B are not assumed to be distributed in any particular way. Their distributions need not be normal or log-normal, for instance. However, in order to estimate q or ρ, it is necessary to know the relation between mortality and the dosage of A and B applied separately, within the limits of sampling error. If, as very often happens, the dosages of A and B can be so trans-

* See Gaddum (1933). In theoretical investigations like the present, normal equivalent deviations arise naturally and are often more convenient than probits, because the addition of 5 to the former to give the latter is arbitrary. A single word, to replace the term 'normal equivalent deviation', would be useful.

formed mathematically that the individual minimum lethal transformed doses are normally distributed, the estimation of q or ρ is facilitated. Transformed measures of dosage have been called *dose-metameters* by Bacharach (1945): that for A need not be the same as that for B. The N.E.D.'s will be linearly related to the metameters for A and B, when A and B are applied separately. Thus, (9) can be used however the resistances are distributed, but it is most convenient when metameters can be found with respect to which the distributions are normal.

The division of the population into the four classes $\alpha\beta$, $\bar{\alpha}\beta$, $\alpha\bar{\beta}$, and $\bar{\alpha}\bar{\beta}$ can be pictured as the section of the bivariate surface into four parts by two vertical planes.

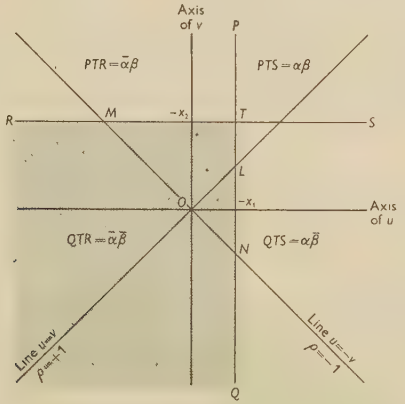

Fig. 3

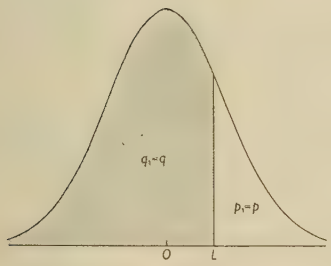

Fig. 3*a*

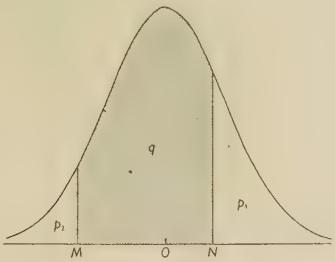

Fig. 3*b*

These planes are parallel to the axes of u and v respectively, i.e. they are at right angles to one another, and cut each other and the base-plane at a point $T \equiv (-x_1, -x_2)$ in the latter. Fig 3 shows in plan this section of the surface. PTQ and RTS represent the vertical planes, so that the volumes under the bivariate surface lying above the sectors PTS, PTR, QTS, and QTR are the proportions of the population in the classes $\alpha\beta$, $\bar{\alpha}\beta$, $\alpha\bar{\beta}$, and $\bar{\alpha}\bar{\beta}$ respectively. Equation (9) defines q as the volume under the bivariate surface above the sector QTR, which is shaded in Fig. 3.

When $\rho = +1$, the bivariate surface shrinks to an univariate curve above the line $u = v$ of Fig. 3. This curve has a standard deviation of $\sqrt{2}$, and is shown in elevation

in Fig. 3 *a*, in which the shaded area represents q, i.e. the area under the curve above the sector QTR. In Figs. 3 and 3 *a*, $-x_2 > -x_1$, i.e. $x_1 > x_2$. When $x_1 \geqslant x_2$, i.e. $p_1 \geqslant p_2$,

$$q = F(-x_1) = q_1,$$

i.e. $p = p_1$. This can be seen from Fig. 3, for whatever the position of T in LP, the portion of the line $u = v$ within QTR is the same. Similarly, when $p_2 \geqslant p_1$, $p = p_2$, so that equations (1) and (2) hold when $\rho = +1$.

When $\rho = 0$, equation (9) becomes simply the product of two single integrals, so that

$$q = F(-x_1).F(-x_2) = q_1 q_2,$$

i.e. $p = p_1 + p_2 - p_1 p_2$, which is equation (3).

When $\rho = -1$, the bivariate surface shrinks to an univariate curve above the line $u = -v$ of Fig. 3. This curve has a standard deviation of $\sqrt{2}$ also, and is shown in elevation in Fig. 3 *b*, of which, as before, the shaded area represents q, the area under the curve above QTR. When T lies to the right of the line $u = -v$, as in Figs. 3 and 3 *b*,

$$p = F(x_1) + F(x_2) = p_1 + p_2.$$

When it lies to the left of the line, none of the area under the curve is in QTR, i.e. $q = 0$ and $p = 1$. Hence equations (4) and (5) hold when $\rho = -1$.

THE DOSAGE-MORTALITY CURVE FOR A MIXTURE OF POISONS TO WHICH THE RESISTANCES ARE LOG-NORMALLY DISTRIBUTED

In this section we consider the dosage-mortality curves for mixtures of poisons when the resistances are log-normally distributed and are either uncorrelated or completely correlated. The dosage-mortality curve for a mixture will differ according to the dose-metameters employed, and as resistances are often log-normally distributed, this has been assumed here.

Both Bliss (1939) and Finney (1947) figured some hypothetical dosage-mortality curves for mixtures of poisons acting independently. Bliss calculated his curves by means of the unsatisfactory equation (6). Finney, in a numerical investigation, gave mixture curves for $\rho = 0$ and $\rho = 1$ only.

Let the relations between N.E.D., y_1 or y_2, and dosage, z, of A and B, be

$$y_1 = \alpha_1 + \beta_1 \log z, \tag{10}$$

$$y_2 = \alpha_2 + \beta_2 \log z, \tag{11}$$

and suppose the parameters* of these equations to be free from error. Let the proportions of A and B in a mixture be π_1 and π_2 respectively, with $\pi_1 + \pi_2 = 1$. Then

$$x_1 = \alpha_1 + \beta_1 \log(\pi_1 z) = \delta_1 + \beta_1 \log z, \tag{12}$$

$$x_2 = \alpha_2 + \beta_2 \log(\pi_2 z) = \delta_2 + \beta_2 \log z. \tag{13}$$

If $\rho = +1$, from (1) and (2), $x = x_1$ or x_2, whichever is the greater. When $\beta_1 \neq \beta_2$, the mixture curve will, therefore, consist of two segments, each a straight line, and

* α and β are parameters here, and are distinct from the classes α, β, etc.

will change its slope abruptly where the lines meet, i.e. where $\log z = (\delta_2 - \delta_1)/(\beta_1 - \beta_2)$. For instance, if $\beta_1 < \beta_2$,

$$x = \delta_1 + \beta_1 \log z \qquad [\log z \leqslant (\delta_2 - \delta_1)/(\beta_1 - \beta_2)],$$
$$x = \delta_2 + \beta_2 \log z \qquad [\log z \geqslant (\delta_2 - \delta_1)/(\beta_1 - \beta_2)].$$

When $\beta_1 = \beta_2$, on the other hand, the mixture curve will be a single straight line, coinciding with that defined by equations (12) or (13) as $\delta_1 \geqslant$ or $\leqslant \delta_2$.

If $\rho = 0$, $x > x_1$ or x_2, so that the mixture curve will lie above the lines defined by (12) and (13). An algebraic investigation, to be described elsewhere by one of us (R.L.P.), has shown that at low doses x tends to the greater of x_1 and x_2, so that the mixture curve is asymptotic to (12) or (13) as $\beta_2 >$ or $< \beta_1$, or, if $\beta_1 = \beta_2$, as $\delta_1 \geqslant$ or $\leqslant \delta_2$; and that at high doses the curve is asymptotic to the line

$$x_u = (\delta_1 \beta_1 + \delta_2 \beta_2)/\sqrt{(\beta_1^2 + \beta_2^2)} + \sqrt{(\beta_1^2 + \beta_2^2)} \log z.$$

Where x_1 is very much greater or very much less than x_2, the lower asymptote approximates well to the mixture curve for N.E.D.'s in the experimental range. The upper asymptote, however, approximates well to the mixture curve only for N.E.D.'s considerably outside the experimental range.

We investigated numerically the mixture curve for $\rho = 0$, and confirmed that the slope of the curve increases from β_1 or β_2, whichever is the less, to a maximum of $\sqrt{(\beta_1^2 + \beta_2^2)}$. Our numerical investigation indicated, as Finney's (1947) did, that the slope of the curve increases smoothly and progressively, though Bliss implied the contrary. Thus, if β_1 is not very different from β_2, and if the lines defined by (12) and (13) intersect not far from zero N.E.D., sets of experimentally observed N.E.D.'s for a mixture should often lie near a straight line of slope about $\frac{1}{2}[\beta_1 + \sqrt{(\beta_1^2 + \beta_2^2)}]$ if $\beta_1 < \beta_2$, or $\frac{1}{2}[\beta_2 + \sqrt{(\beta_1^2 + \beta_2^2)}]$ if $\beta_2 < \beta_1$. If $\beta_1 = \beta_2 = \beta$ and δ_1 is not very different from δ_2, the experimentally observed N.E.D.'s should often fit a line of slope about $(\frac{1}{2} + 1/\sqrt{2})\,\beta$. Numerical examples have supported these conjectures (see Fig. 5).

Parker-Rhodes (1943) stated that the mixture curve is an hyperbola, but it cannot be an hyperbola in the strict sense of the word.

If $\rho = -1$, the N.E.D. for the mixture will be low at low doses, though higher than x_1 or x_2; rise more and more rapidly with increasing log-dosage; become infinite at the finite dose at which $p_1 + p_2 = 1$; and remain infinite at still higher doses. When $p_1 + p_2 = 1$, i.e. $p_1 = q_2$, $F(x_1) = F(-x_2)$, so that $x_1 = -x_2$. Hence the mixture N.E.D. will become infinite at the log-dose at which $x_1 = -x_2$, i.e. $-(\delta_1 + \delta_2)/(\beta_1 + \beta_2)$. The mixture curve is therefore asymptotic to the line $\log z = -(\delta_1 + \delta_2)/(\beta_1 + \beta_2)$.

Fig. 4 shows the mixture curves for $\rho = 0{\cdot}5$, 0, $-0{\cdot}5$, and $-1{\cdot}0$, derived from the lines

$$x_1 = -12{\cdot}5 + 12{\cdot}5 \log z, \quad x_2 = -3{\cdot}500 + 3{\cdot}125 \log z.$$

These lines intersect at (0·96, −0·5), and differ considerably in slope. The mixture curve for $\rho = +1{\cdot}0$ is the line for x_1 at log-doses above 0·96, and that for x_2 at log-doses below 0·96. The asymptote to the mixture curve for $\rho = -1{\cdot}0$ is

$$\log z = 16/15{\cdot}625 = 1{\cdot}024.$$

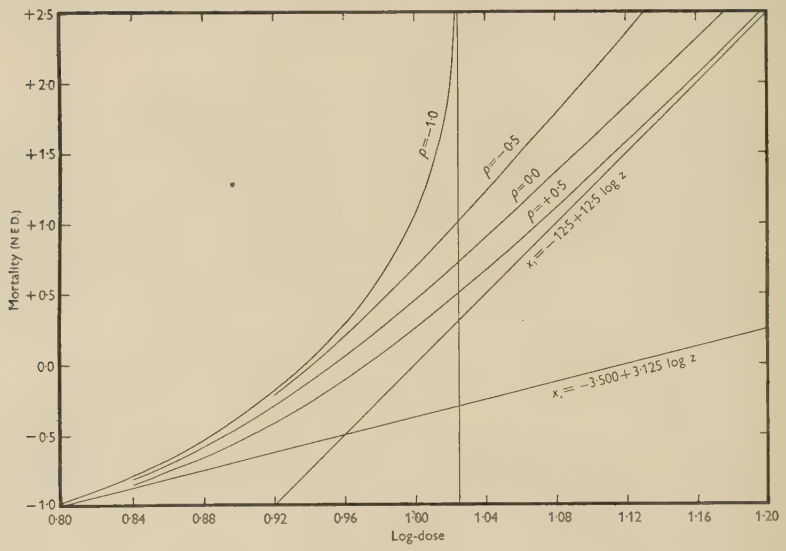

Fig. 4

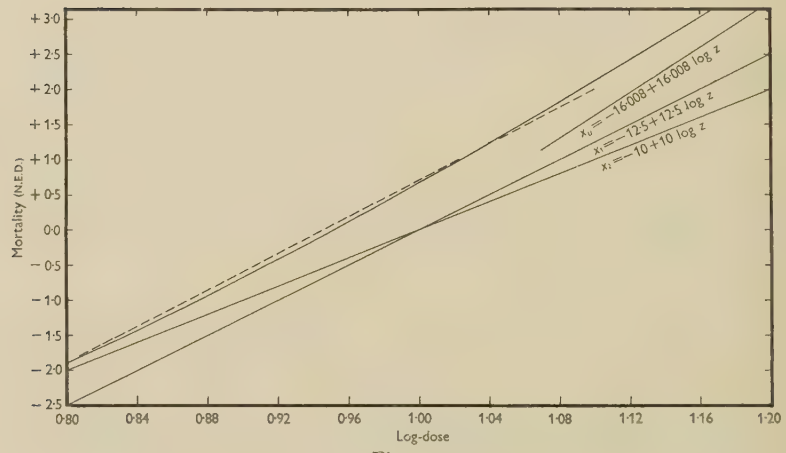

Fig. 5

Fig. 5 shows the mixture curve (continuous line) for $\rho = 0$ derived from the lines

$$x_1 = -12{\cdot}5 + 12{\cdot}5 \log z, \quad x_2 = -10 + 10 \log z.$$

These lines intersect at (1·00, 0) and differ little in slope. The lower asymptote to the mixture curve is the line for x_2, and the upper asymptote, also shown in Fig. 5, is

$$x_u = -16{\cdot}008 + 16{\cdot}008 \log z.$$

Here $\frac{1}{2}[\beta_2+\sqrt{(\beta_1^2+\beta_2^2)}]=13{\cdot}004$, and in Fig. 5 the broken line

$$x=-12{\cdot}3+13\log z$$

should fit experimentally determined sets of mixture N.E.D.'s, agreeing with the predictions mentioned above.

Discussion

An insecticide should be very effective and economical if it contains two poisons, A and B, that act independently, and to which the resistances of the insects are completely and negatively correlated, and log-normally distributed (or otherwise skewly distributed to the right). When the resistances to a poison are so distributed, it requires more of the poison, applied alone, to raise the proportion of insects killed from $(0{\cdot}5+l)$ to $(0{\cdot}5+m)$ than from $(0{\cdot}5-m)$ to $(0{\cdot}5-l)$, where $0\leqslant l<m<0{\cdot}5$. For example, it requires more to increase the proportion from 0·70 to 0·95 than from 0·05 to 0·30. Hence A and B would be very efficiently utilized if A kills the insects more resistant to B, and B kills those more resistant to A. Suppose, for instance, that in (10) and (11), $\alpha_1=-2$, $\beta_1=2$, $\alpha_2=-3$, and $\beta_2=3$. To kill 0·99 of a population of insects would then require 145 units of A alone or 60 units of B alone. Used together, however, 10 units of A and 10 units of B would suffice to exterminate the population: the 10 units of A would kill the half of the population more susceptible to A and more resistant to B, while the 10 units of B would kill the other half, more susceptible to B and more resistant to A. If the correlation in resistance were negative but incomplete, A and B together might still form an effective combination, though it would be less effective than if the correlation were complete.

Bliss (1939) asserted that mixtures of poisons acting similarly could be expected to be more potent than if the poisons acted independently. Finney (1942) thought this was generally so, but later (1947) modified his view, and considered that under some circumstances the reverse could be true. For two poisons, the question can be precisely stated like this: if given doses of poisons A and B applied separately kill respectively proportions p_1 and p_2, and when the poisons act similarly the same doses applied together kill a proportion p_s, and when independently a proportion p_i, is p_s greater or less than p_i? If A and B act similarly, using B in addition to A has the same effect as increasing the dose of A, so that p always exceeds the greater of p_1 and p_2; but the amount to which it does so depends, not only on the values of p_2, but on how rapidly p_1 and p_2 increase with increasing dose of A and B. The rate of increase of p_1 and p_2 may be large or small according to the poisons involved, the method of application, etc. From equations (1)–(5) it is evident that p_i can vary from the greater of p_1 and p_2 to the lesser of (p_1+p_2) and 1. There is, therefore, no general rule to show whether p_s is greater or less than p_i. A similar argument can be followed to show that a mixture of any number of poisons acting similarly can, according to circumstances, be more toxic or less toxic than if they acted independently.

This work has been carried out as part of the programmes of research of the National Physical Laboratory and Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research. We are indebted to Mr E. C. Fieller, of the former Laboratory, for suggesting the use of the bivariate normal correlation coefficient.

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A SPINNING-DISK SPRAYER FOR APPLYING RESIDUAL INSECTICIDES

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(With 5 Text-figures)

The apparatus consists essentially of a spinning disk which throws off liquid, applied to its centre, in the form of small droplets. These are of the same order of size (about 0·1–0·4 mm. diameter) as those produced by knapsack sprayers, but they are more uniform and consistent.

The sprayer can be used to coat various surfaces, up to 1 ft. square, with deposits of insecticides in oil solution or in aqueous suspensions or emulsions. The deposits can be controlled and estimated with a fair degree of precision.

Some rough samples of the spray clouds produced by a knapsack sprayer are recorded.

Introduction

The discovery of persistent synthetic insecticides like D.D.T. has considerably modified laboratory testing methods. Instead of being exposed to direct sprays or mists, the insects are induced to make contact with treated surfaces. There is a need for a convenient and reliable apparatus for applying standard deposits to various surfaces. It should satisfy the following requirements:

(1) Since the physical state of the deposit may affect its toxicity, it is desirable to apply the treatment in a manner feasible in practice. The most suitable methods appear to be by spraying kerosene solutions, aqueous emulsions or dispersions. The apparatus must be able to apply such liquids in the form of sprays and it should produce droplets of the same order of size as those produced by sprayers found efficient in practice (i.e. knapsack pressure sprayers, *not* atomizers). Within this range, the droplet size and the rate of deposit should be adjustable.

(2) For flying insects it may be desirable to conduct the experiments in cages with treated walls. Such cages should be sufficiently large to allow some flying activity. Therefore, the apparatus must be able to treat surfaces at least 1 ft. square.

(3) For obvious reasons, the deposit should be applied evenly.

(4) For convenience, successive applications should be consistent.

After trying other types of apparatus, we took the advice of Mr W. H. Walton and his colleagues of the Chemical Defence Experimental Station, Porton, and employed the spinning-disk principle (which had been developed there). (We understand that a paper describing the physical principles involved will be published elsewhere by W. H. Walton and W. C. Prewett. The disk sprayer has also been used in a somewhat different form for spraying locusts (see Kennedy, Ainsworth & Toms, 1948).

Principles of spinning-disk sprayer

The spray is produced by feeding the liquid insecticide on to the centre of a fast-spinning horizontal disk. The liquid picks up the rotary velocity of the disk and spreads outward under the influence of centrifugal force. Finally it is projected from the periphery in the form of droplets. The direction taken by the liquid particles after they leave the disk is presumably a resultant between the radial and rotary velocities attained at the last moment. Apparently the latter is very large in comparison with the former, because the path taken by the droplets is nearly tangential.

The spray clouds consists of two kinds of droplet; the bulk of the liquid forms relatively large droplets of fairly uniform size, but there is also a proportion of much smaller droplets, produced as satellites during the formation of the larger ones. The two types of droplet are normally separated by air resistance, which slows up the fine satellites very rapidly, while the larger uniform droplets travel much farther from the disk.

Walton and Prewett have shown that the size of the main (large) droplets depends upon different variables as follows:

$$d = K\frac{1}{\omega}\sqrt{\frac{T}{D\rho}},$$

where d = mean drop diameter, ω = rotary velocity (radians per sec.), T = surface tension of liquid, ρ = density of liquid, D = diameter of the disk. K is a constant of the order of 3·8

Details of insecticide applicator

The sprayer consists of a $\frac{1}{8}$ h.p. electric motor, on the spindle of which various brass disks can be mounted to spin in the horizontal plane, above the motor. The disks are polished and the edges bevelled underneath at an angle of 45°. The liquid insecticides are fed from a constant-level burette, of capacity about 150 c.c., mounted with the tip close to the centre of the disk. The maximum rate of delivery, with the tap fully open, is about 35–40 ml./min. (see Fig. 2). The disk is surrounded by a metal shield with a removable lid, which is pierced to allow the tip of the liquid feed to approach the centre of the disk. This shield traps the spray except for about one-ninth of it which is allowed to escape from a slit 2 in. long by $\frac{1}{2}$ in. high. The spray liquid caught in the shield drains out down a metal tube fixed to the bottom. To prevent oil running into the motor down the spindle which passes through the shield, a collar is fitted around the spindle hole in the shield, extending up almost to the under surface of the disk.

A coarse resistance is wired into the disk motor circuit to facilitate a wide range of disk speeds, and slight variations of speed are controlled by a fine adjustment resistance placed between the coarse resistance and the motor. In order to get very low disk speeds, it was found necessary to introduce a lamp holder into the circuit

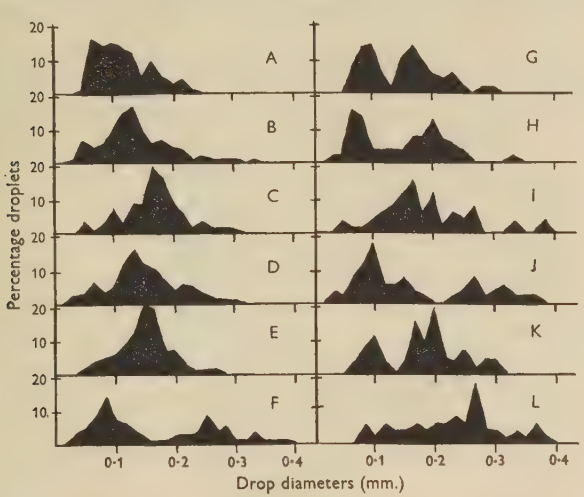

Fig. 1. Drop-size distribution curves obtained with a knapsack sprayer. Key:

Nozzle	Pressure (lb./sq.in.)	Water	Kerosene
$\frac{1}{32}$ in.	75	A	G
	50	B	H
	25	C	I
$\frac{1}{16}$ in.	75	D	J
	50	E	K
	25	F	L

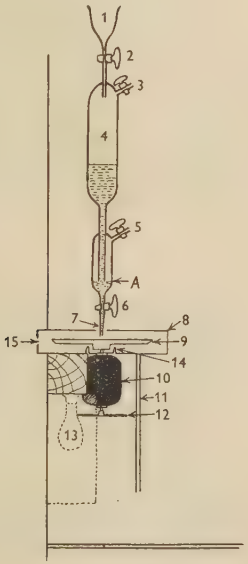

Fig. 2. Showing details of constant level burette and spraying unit. 1, filling funnel; 2, 3, 5, 6, one way taps; 4, reservoir; 7, delivery nozzle; 8, metal shield with lid; 9, brass disk with bevelled edge; 10, motor; 11, drain pipe; 12, stroboscope disk; 13, neon lamp; 14, metal collar to prevent leakage into motor; 15, spray aperture in disk shield. Level *A* represents the constant column of liquid. When filling the burette, taps 2 and 3 are opened, and taps 5 and 6 are closed. When the burette is in operation taps 2 and 3 are closed and taps 5 and 6 are opened.

in which electric light bulbs of suitable wattage could be fixed to give the required resistance.

The speed of rotation of the disk is determined by using a stroboscope disk fitted to the lower end of the disk motor spindle. The stroboscope disk is divided into

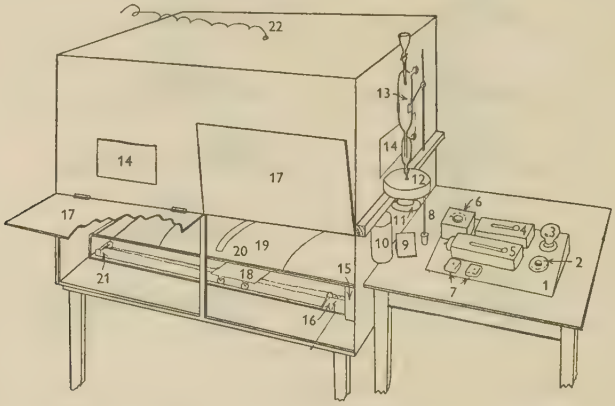

Fig. 3. General view of sprayer. 1, switch board; 2, fine adjustment resistance controlling disk motor speed; 3, resistance bulb in disk motor circuit; 4, coarse resistance controlling disk motor speed; 5, coarse resistance controlling track motor speed; 6, coarse resistance controlling disk motor speed; 7, switches; 8, drain pipe from disk shield; 9, mirror for viewing stroboscope disk; 10, metal reflexion shield enclosing neon lamp for illuminating stroboscope disk; 11, stroboscope disk; 12, metal shield enclosing spinning disk; 13, constant level burette; 14, glass windows in walls of cabinet; 15, wooden housing for track motor; 16, haulage gear for trolley; 17, hinged doors on side of cabinet; 18, trolley mounted on rails; 19, curved perspex shield; 20, slot cut in perspex shield; 21, pulley mechanism for haulage chain; 22, lead to cabinet lamp.

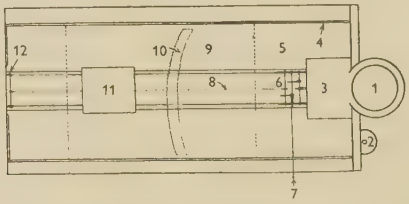

Fig. 4. Spinning disk apparatus as seen from above. 1, spinning disk; 2, neon lamp illuminating stroboscope disk; 3, wooden housing for track motor; 4, wooden supporting frame for perspex shields; 5, immovable perspex shield; 6, haulage gear for trolley; 7, gear release rod for reversing trolley; 8, continuous meccano haulage chain; 9, sliding perspex shield; 10, slot cut in perspex shield (1½ in. wide); 11, trolley mounted on rails; 12, pulley mechanism for haulage chain.

concentric rings, each of which is divided into equal numbers of black and white bands. The disk is illuminated by a neon lamp (which extinguishes more rapidly than a filament lamp) operated from the 50 cycle a.c. mains supply. The a.c. current

illuminates the disk every $\frac{1}{100}$ sec., i.e. $\frac{1}{6000}$ min. Therefore, if the disk revolves once in this time, a single black bar will always be shown at the same place. At 3000 r.p.m. two black bars, and at 2000 r.p.m. three bars will change places, and so forth; hence, certain primary speeds are indicated by apparently stationary patterns. Some intermediate and slower speeds are shown by fainter patterns produced by partial overlapping of the illuminated positions. According to the phase illuminated, the various rings will indicate spinning speeds varying from 400 to 6000 r.p.m.

The spray which is allowed to escape from the shield is projected into an enclosed chamber, to mitigate the effects of draughts. The spray droplets travel out in a fan-shaped path, gradually losing their horizontal velocity owing to air resistance, and begin to fall earthwards. They are allowed to fall $1\frac{1}{2}$ ft. on to the target after which they have lost most of their radial velocity and are falling almost vertically at a speed dependent upon their weight and size. They fall on a fairly narrow band in the form of a shallow arc about $1\frac{1}{2}$ ft. wide. Surfaces to be sprayed are drawn through the band of spray on a trolley mounted on rails. The haulage gear is constructed of 'Meccano', and is operated by an $\frac{1}{8}$ h.p. electric motor fed from the a.c. mains supply. Incorporated in the circuit is a coarse resistance to control and alter the speed at which the trolley passes through the band. The haulage winding is geared down to give a track speed ranging from $\frac{1}{3}$ to 1 m./min.

Calibration and performance of apparatus

(1) *Sizes of droplets*

(*a*) *Methods of measurement*

The numbers of droplets falling in different zones were simply determined by adding a trace of dye to the liquid and counting the splash marks.

Droplet sizes were measured either (i) by impaction on magnesium oxide coated glass slides and measurement of the crater diameter (May, 1945), or (ii) (aqueous sprays) by catching the droplets in shallow troughs filled with a solution of vaseline in heavy mineral oil (about 1:3). Droplets of water form spheres in this viscous liquid and, falling to the bottom very slowly, they can be measured with a micrometer eyepiece.

(*b*) *Results*

(i) *Effect of rate of delivery on mean drop size.* The mean diameter of water droplets produced by the disk spinning at 2000 r.p.m. was determined by the vaseline-oil method, for different rates of delivery of the liquid on to the disk.

Rate of delivery (ml./min.)	Mean drop diameter (mm.)
2	0·296
18	0·322
35	0·326

It is evident that droplet size is not greatly affected by small differences in the rate of delivery.

(ii) *Effects of size of disk, spinning speed, type of liquid, etc.* The effect of certain variables on the mean droplet diameter are set out in Table 1.

It will be seen that our data are consistent with the formula suggested by Walton and Prewett.

For routine use, droplets of about 300 μ seem to be suitable; and these may be produced by the 14 cm. disk spinning at 2000 r.p.m., or the 10·5 cm. disk spinning at 2000–3000 r.p.m.

Table 1. *Effects of variables on droplet size produced by spinning-disk sprayer*

Liquid	Density (g./c.c.)	Surface tension (dynes/cm.)	Disk diameter (cm.)	Revolutions per min.	Droplet size (μ)	$K = \omega d \sqrt{\frac{D\rho}{T}}$
Water + 5 % Teepol	1·0	30·8	7·5	1200	640	3·97
				1500	500	3·88
				2000	375	3·88
			10·5	1200	560	4·11
				1500	450	4·13
				2000	300	3·67
			14·0	1200	450	3·81
				1500	350	3·71
				2000	270	3·81
Odourless distillate	0·78	25·6	7·5	1200	730	4·38
				1500	560	4·21
				2000	410	4·10
			10·5	1200	610	4·34
				1500	480	4·26
				2000	350	4·15
			14·0	1200	505	4·14
				1500	400	4·10
				2000	320	4·38
White oil P31	0·86	28·2	7·5	1200	800	4·81
				1500	625	4·69
				2000	470	4·71
			10·5	1200	640	4·55
				1500	520	4·62
				2000	390	4·62
			14·0	1200	550	4·51
				1500	450	4·62
				2000	350	4·79

(iii) *Droplet-size distribution and spray-band width.* These data were obtained with various spray liquids, using the 10·5 cm. disk spinning at 2000 r.p.m. and a liquid delivery rate of 20–30 c.c./min. The results are best shown graphically, as in Fig. 5.

With odourless distillate the total range of drop size lies between 0·14 and 0·2 mm. radius, and the width of the band is 4 in. between 20 and 24 in. from the disk. Also approximately 97 % of the total number of drops are between 0·16 and 0·18 mm. radius, the greater proportion of which fall in a 2 in. band between 21 and 23 in. from the disk. Teepol (5 %) also forms a distinct band with a drop-size range from 0·13 to 0·19, but the band covers a distance of 5 in. from 22 to 27 in. from the disk.

No band is apparent with distilled water and there is a fairly even distribution of droplets in all zones.

It was evident from these results that formation of a well-defined band was due to production of droplets of uniform size; this only occurred with liquids of low surface tension. According to Mr Walton (personal communication) failure to produce uniform droplets (at low rates of liquid delivery) was due to imperfect wetting of the brass disk. However, it was found, in practice, that most insecticidal fluids had low surface tensions. [*N.B.* Oils are all low, and aqueous suspensions or emulsions always contain surface-active wetting agents.]

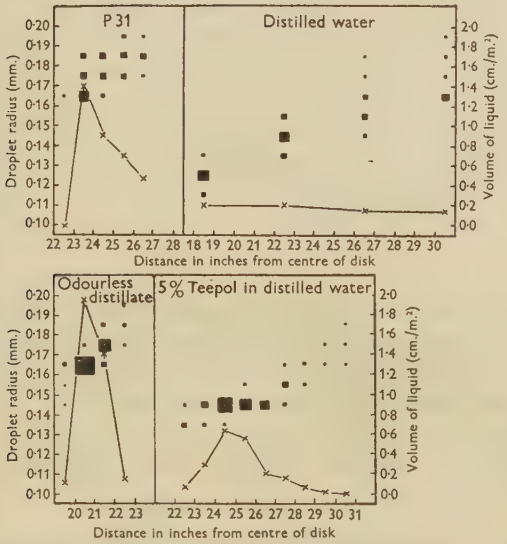

Fig. 5. Showing the distribution of the size and number of drops throughout the band when spraying 0·5 ml. of liquids of different surface tensions at a disk speed of 2000 r.p.m. The black squares represent the relative number of drops/in.² of each size for each zone. The points on the graph represent the volume of liquid deposit in cm./in.² at varying points throughout the band.

(iv) *Lateral distribution in the band.* 0·5 ml. of 5% Teepol was sprayed on to a stationary piece of squared paper at a disk speed at 2000 r.p.m. so as to produce a band of spray droplets approximately 1 ft. in length. A count was made of droplets per sq.in. throughout the length of the band. The figures for successive sq.in. were: 230, 226, 200, 201, 201, 229, 211, 205, 222, 211, 200, 208 and 215; mean 220. It will be seen that there is not very much lateral variation in the number of droplets. Samples of the droplets caught on magnesium oxide slides during this test gave a droplet size of 0·14–0·15 mm. radius.

(2) *Total deposits*

(*a*) *Methods of measurement*

Total deposits of liquid per unit area were estimated roughly by rapid weighing of test papers before and after spraying, using a torsion balance. Two methods were employed for estimating the insecticide deposited:

(i) A dye was included in the spray fluid and later extracted from sprayed test papers and estimated colorimetrically. This gave an indirect estimate of the insecticide present.

(ii) D.D.T. or *gamma* benzene hexachloride could be estimated chemically by the following procedure. The insecticide was extracted in benzene from sprayed test papers of adequate size. The solvent was largely boiled off and the labile chlorine liberated by hydrolysis with alcoholic potassium hydroxide. The solvent was again removed and the excess alkali neutralized with nitric acid. The chloride was then determined by adding a known excess of silver nitrate and back-titrating with potassium thiocyanate using ferric nitrate as an indicator. Using N/200 solutions, the end-point was discernible with an excess of about 0·05 ml. of KSCN which corresponds to an error of 0·08 mg. of D.D.T. or 0·02 mg. of Gammexane; i.e. 8 or 2% respectively of 1 mg. of insecticide.

(*b*) *Results*

For standard experiments all spraying was done with a constant track speed of 1 m. in 45 sec., the actual deposit of insecticide being determined by the concentration of the spray fluid. This ensured that approximately the same amount of liquid was deposited per sq.cm. in all the experiments.

Weights of liquid per sq.cm. were determined by spraying small pieces of test paper and rapidly weighing them on a torsion balance with the following results:

Spraying odourless distillate

	Weight of liquid (mg.)	
1.	20·6	Using 25 cm.^{2} of paper.
2.	20·2	
3.	20·3	Disk speed 2000 r.p.m.
4.	20·4	
5.	20·4	Mean weight = 20·4 mg.
6.	20·5	

Test papers 300 cm.^{2} were sprayed with a 5% solution of D.D.T. in dyed odourless distillate and the amount of insecticide estimated by the colorimeter method and also by chemical analysis with the following results:

Colorimetric estimation D.D.T. (mg./cm.^{2})	Chemical estimation D.D.T. (mg./cm.^{2})
0·027	0·028
0·029	0·028
0·028	0·028
0·029	0·026
0·029	0·028

It is possible to apply a definite and known amount of insecticide to a surface and to repeat such a deposit to within 5% error at the most.

Tests with field-spraying equipment

Ordinary knapsack sprayers have been found most satisfactory for applying residual insecticides to walls and similar surfaces. It was considered desirable to make some rough estimates of the drop-size distributions of the spray clouds produced by equipment of this type. Most of the tests were done with the Four Oaks Kent model pressure sprayer; a few tests with a Ross type, continuous action sprayer gave similar results. Both types produce a spray by ejecting liquid in the form of a rotating hollow cone. The liquid sheet expands, and becomes thinner on receding from the nozzle and breaks up into threads which finally form droplets. The dispersal of these droplets is complicated by different circumstances. Their origin in a rotating cone gives them a variety of initial directions, though all have a general movement away from the nozzle, their original velocity depending on the pressure employed. The larger droplets have more momentum relative to their air resistance (surface area) than the small droplets; consequently they tend to travel farther in still air. However, the movement of the cloud of spray droplets soon produces a forward air current which carries along the finer droplets. Therefore, very fine and coarse droplets travel farthest.

The tests were done in a small room with draughts excluded as far as possible. The spray was projected horizontally at $2\frac{1}{2}$ ft. from the floor and samples of falling spray were collected at floor levels, 1, 2 and 3 ft. in front of the nozzle. After consideration of the individual results from the samples at different distances from the nozzle, drop-size distribution from all three zones were combined to assess the characteristics of different spray clouds.

Results

Fig. 1 gives typical droplet-distribution curves of spray clouds from $\frac{1}{16}$ in. and $\frac{1}{32}$ in. nozzles, using water or kerosene and different pressures. It will be seen that the curves are very irregular owing, perhaps, to the rather rough experimental conditions. However, the data are adequate to indicate the general sizes of droplets produced, which mainly lie between 0·1 and 0·4 mm. diameter. The average droplet sizes and fluid outputs at different pressures are given in Table 2.

Table 2. *Showing mean drop sizes and delivery rates with the Kent type sprayer*

Spraying pressure (lb./sq.in.)	Water				Kerosene			
	Mean drop size (mm. diam.)		Spray rate (c.c./sec.)		Mean drop size (mm. diam.)		Spray rate (c.c./sec.)	
	$\frac{1}{16}$ in.	$\frac{1}{32}$ in.	$\frac{1}{16}$ in.	$\frac{1}{32}$ in.	$\frac{1}{16}$ in.	$\frac{1}{32}$ in.	$\frac{1}{16}$ in.	$\frac{1}{32}$ in.
75	0·147	0·119	18·30	5·60	0·163	0·148	26·50	7·80
50	0·151	0·141	15·20	4·60	0·175	0·147	20·50	6·60
25	0·168	0·163	11·50	3·30	0·290	0·185	15·40	4·40

The work was carried out in the London School of Hygiene and Tropical Medicine, Department of Entomology, Director: Prof. P. A. Buxton, C.M.G., F.R.S. It was partly financed by a grant to one of us (H.C.M.P.) by the Medical Research Council.

We wish to record our appreciation of the work of Mr N. F. Wilson who has shown diligence and resource in the construction of the apparatus. The constant level burette was very kindly made for us by Mr C. N. Davies, who also offered valuable suggestions.

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PASTURE IMPROVEMENT AND THE CONTROL OF SHEEP TICK (*IXODES RICINUS* L.)

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The sheep tick is confined almost entirely to rough, poor hill and moorland pastures. Agricultural improvement of these pastures involves (among other things) reduction in the depth of the vegetation layer and partial or complete destruction of the moisture-holding underlying 'mat' of decaying vegetable matter. Assessment of the tick populations on improved and on comparable control areas showed a reduction in tick population following upon pasture improvement. But a fairly considerable degree of improvement is required to bring about a satisfactory reduction. Ticks were not completely extirpated even by ploughing. It is probable that pasture improvement affects tick population mainly by reducing cover and thus the humidity.

Unfortunately, effective pasture improvement is not economic on hill and moorland grazings in general. Such improvement as is practicable could only be a supplement to other control measures.

Since the sheep flock supports almost all the female ticks on a pasture, control is most likely to follow from the development of an efficient acaricidal sheep dip.

Introduction

The sheep tick, *Ixodes ricinus* L., is a major pest on British hill farms. It has four stages: egg, larva, nymph and sexually mature adult; and development takes place on the ground. A blood meal is necessary at the larval, nymphal and adult female stages; the male rarely feeds, but it must be on a host to meet the female. Over Britain as a whole, spring is the main host-seeking season; additional autumn activity is confined to western areas (Milne, 1945*a*, 1947*a*). In its various stages, the tick spends a total of about 3 weeks on hosts; the remainder of its 3 years' existence is passed on the ground (MacLeod, 1932; Campbell, 1946).

On the hill and moorland pastures of northern England only a small proportion of ticks in the ground population is fed by the wild fauna; the great majority are parasitic on sheep (Milne, 1948*a*). A previous paper (Milne, 1945*b*) dealt with the possibilities of control by acaricidal treatment of farm stock; the present paper examines the effects of treatment of land. MacLeod (1939) discussed this method but carried out no field trials.

In Britain, the sheep tick is practically confined to rough, poor hill and moorland pastures; judging by population density, the better the pasture, the fewer the ticks; indeed they are absent entirely from good lowland pastures (Milne, 1944).

A rough hill pasture is generally dominated wholly or in parts by bents or bracken or heather. The present paper does not deal with the heather type. A description of

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the rough bent and bracken pasture is given by Stapledon (1937): it is characterized by a rank, dense, surface vegetation with an underlying layer of mat or litter and moss. This provides, over much of the pasture area, a ground environment of 100% humidity even in summer and so makes possible the survival of ticks, which are highly susceptible to desiccation (see Milne, 1948*c*).

PLOTS AND TREATMENTS

In this exploratory investigation, several pairs of neighbouring plots, one treated and one control, were selected at scattered points on Northumbrian hill grazings. The site chosen for each pair was superficially uniform and all areas were rough pasture with a thick underlying layer of mat or litter. The plots were usually unfenced and each covered 1–2 acres. A number of pasture-improvement methods were tried but only the more drastic—harrowing, discing and ploughing—are worth mentioning in detail. With two exceptions, the trials were made at Hethpool Farm, Northumberland.

Hethpool plots

Table 1 indicates the experimental scheme. Plot pairs 1–3 were on one pasture and pairs 4–7 on another neighbouring pasture.

Set I consisted of plots A and B (pair 1) and plots C and D (pair 2). A and B were unfenced, in bracken with intermingled bents and other grasses, on a dry slope. In the summers 1941–3 the bracken of plot B was cut and removed, and 2 tons per acre ground limestone applied in October 1941. The plot was disced four ways (at right angles and on both diagonals) in August 1942.

Pair 2 (plots C and D) was similar to pair 1; plot D received the same treatment as plot B except that it was not limed.

Set II. The vegetation on plots E and F (pair 3), which were unfenced and on a less dry and gentler slope, was chiefly *Nardus stricta* with some *Molinia caerulea.* In mid-January 1944, plot F was harrowed three ways with a heavy pitchpole harrow, and the torn-up mat and rough grass were carted off soon after. An application of 2 tons burnt lime and 10 cwt. slag per acre was made during the first half of March 1944.

Pairs 4 and 5 were similar to pair 3. Plots H and L were treated as plot F, except that H had no lime and L no slag.

Set III. Pairs 6 and 7 were in bent (chiefly *Nardus stricta*), with some intermingled rush (*Juncus articulatus*), on almost flat ground. In January 1943, plots N and P were ploughed and disced. As ordinary ploughing of rough hill pasture does not bury the thick vegetation layer, a heavy Jumbo-trac plough was used, giving a furrow 14 in. wide and 8 in. deep in the soil. This turned the vegetation under, and a heavy discing immediately afterwards filled in most of the crevices. No nearer approach to burying and sealing off the tick microhabitat is possible in farming practice. In April 1943, 2 tons per acre burnt lime, and in May 1943,

5 cwt. per acre slag, were harrowed in, followed by seed of a good grass mixture and rape. The treated plots were fenced *after* ploughing so that no rough tick-infested margins were enclosed. Up to June 1944 they were grazed by sheep freed of ticks before entry; after that they were always open to the sheep and cattle of the surrounding area containing their control plots (as were plots B, D, F, H and L right from the time of treatment).

Other plots

Two farms, on which part of a hill pasture had already been ploughed, provided a useful comparison with the trials of Set III. The farmers had taken no special precautions (as at Hethpool in the early stages) against stock subsequently carrying ticks to the reseeded land.

High Thorneyburn. The area observed was about 50 acres of hill pasture composed of very matted *Nardus stricta*; 7 acres were reseeded and fenced. The sequence of treatments was:

1940 Ploughed, limed, slagged (including superphosphates); seeded with rape; grazed with sheep.
1941 Again ploughed; rape and seeds (grass); grazed with sheep.
1942 Grazed throughout the year with cattle and sheep.
1943 Grazed until the end of May, then saved for hay.
1944 Farmyard manure in spring; grazed by sheep until after the tick count.

Dykenook. Of 45 acres of very rough benty hill pasture, 25 acres were fenced and treated as follows:

1943 Ploughed in spring, except for a few ditches left rough; limed, slagged and seeded with rape and grass; grazed by sheep in autumn.
1944 Grazed by sheep.

Experimental results

Effects of treatment on vegetation

Discing. On plots B and D about half of the mat and litter was torn up by the discing in 1942, and much of the remainder was loosened, the consequent aeration accelerating its decay. The lime was thoroughly incorporated with the top $1\frac{1}{2}$–$2\frac{1}{2}$ in. of soil on plot B by the end of 1942. By 1943, bracken on plots B and D was so sparse and weak that it was negligible; the vegetation layer as a whole was much shallower and the mat thickness was 0–0·7 in., compared with 2·2–4·8 in. in plots A and C. The better types of grasses and wild white clover had been encouraged especially on the limed plot B. Both cattle and sheep showed marked preference for the herbage on plots B and D, especially B. That is, stock density was greater on these plots than on their controls. After 1943 the treated plots gradually deteriorated, although the rate of deterioration was reduced by the grazing preference of the stock. In 1947, plots B and D were still relatively short-grassed, with a negligible amount of bracken.

Harrowing. Plots F, H and L also showed improvement and were preferred by stock. Where lime only was used after pitchpoling the pasture became more open, probably because of the acceleration of the decomposition of the mat, but the better indigenous grasses and wild white clover were less vigorous than where slag alone had been employed. The greatest improvement occurred where both slag and lime were used. Being on damper ground, plots F, H and L deteriorated more quickly than B and D but had not, by 1947, reverted to their pre-treatment level.

Ploughing. Despite the thorough initial cultivation, plots N and P deteriorated rather rapidly, partly because of difficulties in grazing management, and partly because of poor drainage. *Juncus articulatus* reappeared in 1944 and, freed from the former degree of competition with bents, it was becoming dominant in 1947. After the gates were left open (from July 1944 onwards), stock from the surrounding areas lay much on these plots. The condition of the herbage in 1947 was better than in 1942.

On the plots at High Thorneyburn and Dykenook, the reseeded areas were excellently managed. Practically no deterioration occurred and the herbage remained fine, close-grazed, open and free of mat, in sharp contrast to that of the untreated area.

Effect of pasture improvement on tick population

Methods of estimation

Methods of tick estimation have been previously described (Milne, 1943, 1946). In this investigation, relative population densities were assessed by two methods. In the 'blanket' method, sets of two or three 50 yd. drags were taken almost simultaneously on each plot of a pair on 20–30 days spaced over the spring tick season; in the second method, female tick counts were made on similar groups of sheep grazing on the plots under examination.

Initial effect

Counts made before the pasture-improvement trials showed that the pairs of plots had tick populations of similar density. Relative nymphal populations on two pairs of contiguous 1-acre plots were: pair (i), 5·6, 5·1; pair (ii), 6·3, 6·7 (means per drag).

Certain experiments at Hethpool (not reported above) involved a comparatively slow rate and small degree of pasture improvement. These consisted of either annual cutting and removing of rank growths in their prime or, what amounts to the same, inducing closer grazing by dressings of ground limestone or slag or common rock salt. Both methods lessen roughness and hence decrease mat production. But the process is slow. After 2 years mat was little reduced and no striking effect on tick population was apparent. But with more drastic methods (discing, harrowing, ploughing) the picture was different.

The tick counts assembled in Table 1 show that in the first season after treatment the sample means of nymphal populations were lower on the treated plot than on

the control in all the pairs included in the experiment. The means have a fairly large standard error but, except in pair 4, the differences are significant. The method of estimation may underrate the differences because improvement leads to a more level herbage surface and this permits more intimate contact with the blanket in dragging; hence the improved pasture probably yields a higher proportion of its active ticks than the control.

TABLE 1. *Relative nymphal populations on treated and control plots at Hethpool*

Populations given as mean ticks per drag in 60 drags from each plot.

Set	Pair	Plot	Cultivation	1943	1944	1945	1946	1947
I	1*	A	Control	16·4	20·3	14·1	9·9	13·5
		B	Disced August 1942	0·9	5·5	6·5	5·4	8·8
	2	C	Control	14·7	25·6	20·7	11·1	—
		D	Disced August 1942	3·6	6·2	7·4	2·8	—
II	3	E	Control	—	19·2	12·1	4·6†	9·3‡
		F	Harrowed January 1944	—	8·9	6·4	4·0	12·6
	4	G	Control	—	3·6†	6·2	4·0†	7·0†
		H	Harrowed January 1944	—	3·2	3·4	5·8	8·6
	5	K	Control	—	4·6	5·4	2·1†	—
		L	Harrowed January 1944	—	2·5	2·7	2·0	—
III	6	M	Control	—	5·4	6·1	3·4	8·7†
		N	Ploughed January 1943	—	0·8	2·5	1·7	6·7
	7	O	Control	—	1·8	6·5	3·1	4·1†
		P	Ploughed January 1943	—	0·5	2·2	1·7	5·0

* In spring 1941 and 1942, the sample mean of population was slightly higher in plot B than in plot A, but the difference was not significant in either case.

† No significant difference between members of a pair; in all other pairs the means are significantly different.

‡ Note: control significantly lower than treated plot; these 'significances' take account of sampling variation only.

TABLE 2. *Relative yields per acre of female ticks on ploughed and control areas of rough hill pasture in* 1944

These have been calculated from simultaneous counts on groups of 20 sheep. The sheep were on the control areas from the beginning of the spring tick season, and on the ploughed areas 2–3 weeks before counts were made in late April or early May.

	Female ticks per acre (relative numbers)	
	Ploughed area	Control area
Hethpool	1	5·3
Dykenook	1	12·9
High Thorneyburn	1	23·1

Table 2 data for Hethpool and Dykenook show that results for female ticks on sheep are similar to those for nymphs on the ground. Here again the actual difference in ground density is probably wider than observed, since a higher proportion of ticks will be swept up under the heavier stocking conditions of the reseeded areas (Milne, 1945*c*).

Applying the test for the significance of the mean of a unique sample (Fisher, 1941, § 24) to the 1944 data in Table 1, it is found that the results for treatment are significant at $P = < 0{\cdot}05$. The design of the experiment, however, does not permit of differentiation between the values of the respective treatments. But the work did show that treatment must be fairly drastic to have any considerable immediate effect. Even the very drastic ploughing on plots N and P fell appreciably short of extirpating the ticks initially despite precautions (see p. 371) against stock bringing engorged ticks between treatment and the first sampling. The wild fauna contribution of ticks would be negligible.

Persistence of effect

At High Thorneyburn, where pasture quality was maintained by good management, tick population was still very low, relative to the control, 4 years after initial treatment (Table 2). Fencing may have played a small causal part in this result (see below). At Hethpool the rate of pasture deterioration was, in descending order, (i) plots B and D, (ii) N and P, (iii) F, H and L. (Although not ploughed, plots B and D were favoured by drier soil than N and P.) Observed mean populations did not approximate to those of the controls until after (i) > 5, (ii) 3 and (iii) 2 years respectively in these groups (see Table 1). Thus the reduction of effect on ticks was generally correlated with the progress of pasture deterioration.

These results require some qualification. Equal *active* tick populations would signify no reduction effect only if the numbers of engorged precursors on treated and control plot had also been equal. But an *unfenced* treated plot attracts stock so that they bring ticks from the surrounding area, i.e. more engorged ticks are dropped on a treated plot than on its control. Hence treatment was subjected to a more stringent test on Hethpool and approximation of observed means probably does not mark the end of treatment effect there.

Grazing preference may also affect other results in Table 1. For instance, liming not only enhances the direct effect of cultivation but also promotes the growth of 'sweeter' herbage, more attractive to stock. The latter effect lasts for some time and this may explain why plot F (limed 1944) had a higher tick population than its control in 1947.

Degree of reduction

In addition to the stocking complication noted above, the trials have the disadvantage that they are too narrow in scope to give the precise degree of population reduction to be expected from any treatment. But fairly drastic pasture improvement measures apparently gave rise to reductions of tick population amounting to 50–95%, assuming that populations in a plot pair would have remained approximately equal in the absence of treatment. Using Fisher's method (1946, § 26·2), the true population ratio A/B for 1943 (Table 1) may be said to lie between 16 and 130, but non-normality of the distributions makes these limits somewhat untrust-

worthy. For satisfactory assessment of the effects of pasture treatment on tick populations it would be necessary to follow the standard practice of experimental design used in other branches of agricultural research. In such designs, stocking would be the major difficulty.

Effects on different tick stages

The totals of adults and nymphs for all plots listed in Table 1 were: control plots, 1382 adults and 15,810 nymphs; treated plots, 1066 adults and 7698 nymphs. (Males formed 47% of the adults.) Thus there were, for every one adult, 11·4 nymphs on the control plots but only 7·2 on those treated. The detailed figures are given in Table 3, which shows that the nymph/adult ratio is lower on the treated than on the control plot in 24 out of 28 pairs. The ratio is lower on treated plots even where the active nymph populations have approximated to or exceeded those of the controls (see, for example, pair 3 among results for Sets II and III for 1946 and 1947 in Tables 1 and 3). This supports the contention (above) that Table 1 data do not show the end-point of pasture-treatment effect in Sets II and III. The higher mortality of the nymphs is probably the result of their smaller (Lees, private communication) resistance to desiccation.

TABLE 3. *Nymph/adult ratios on treated and control plots* (*cp. Table* 1)

Set and treatment	Pair	Plot	Treatment or control	1943	1944	1945	1946	1947
I. Discing	1	A	C.	12·6	20·3	14·1	3·8*	15·0
		B	T.	4·5	13·8	9·3	3·9	14·7
	2	C	C.	14·7	15·1*	18·8	3·8	—
		D	T.	6·0	20·7	9·2	3·5	—
II. Harrowing	3	E	C.	—	24·0	15·1	4·4	25·4
		F	T.	—	14·8	8·0	2·7	7·5
	4	G	C.	—	21·2*	24·8	3·1	16·8
		H	T.	—	48·0	22·7	2·5	16·6
	5	K	C.	—	46·0	27·0	3·3	—
		L	T.	—	19·2	3·9	3·1	—
III. Ploughing	6	M	C.	—	77·1	16·1*	2·9	32·6
		N	T.	—	47·1	16·7	2·0	9·1
	7	O	C.	—	105·9	28·3	4·1	13·7
		P	T.	—	15·1	9·6	1·8	12·0

* Value for treated plot higher than for control in only four cases among 28.

DISCUSSION

Ixodes ricinus requires a considerable amount of ground-vegetation cover for protection from predators, and especially from lethal conditions of humidity and perhaps temperature (Milne, 1948*c*). Pasture improvement reduces cover and hence tick population.

Tick control is mainly a problem of hill and moorland pastures, and a major difficulty is the need to control ticks over the whole of the farm. The trials detailed

in this paper suggest that drastic pasture improvement would give a large measure of control within the treated area, provided that pasture quality was maintained.

While drastic pasture improvement over the whole of a farm of 800–5000 acres of rugged country is obviously impracticable, it might be feasible to cultivate and reseed certain portions. If the improved areas were unfenced then the total tick population of the farm would be reduced (1) by killing of ticks already on the improved areas, (2) by killing of those brought in on stock from the surrounding untreated areas. Unfortunately, these improved areas could never amount to more than a very small fraction of the farm. Moreover, they would require to be fenced as otherwise they would be overgrazed and become heavily infested with internal parasites. Even fencing would not obviate the digestive troubles that would ensue when stock were (necessarily) moved from unimproved to richer herbage and vice versa. All things considered, it appears unlikely that drastic improvements of minor portions of a hill farm can be of much practical value.

There remain the possibilities of less drastic pasture improvement. Tick population is denser on the rougher parts of a farm, usually the valley floors and lower slopes (Milne, 1946). These are also the parts presenting least difficulty to agricultural operations. Methods applicable to these areas would be: (1) bracken eradication, (2) harrowing of bents, (3) slagging, and (4) liming. Methods applying more or less over the whole farm are: (5) clearing of choked sheep drains, (6) burning and (7) mixed grazing; sheep take only the finer herbage while cattle include the coarser grasses which, unchecked, give rise to the roughness and mat favouring ticks. Bracken eradication, manuring, drainage and hill cattle are among items covered by State subsidy or grant (see the Hill Farming Act of 1946), but there are limits to practicable expenditure on these items and the degree of improvement obtained would fall far short of that produced by more drastic methods. Hence, although total tick population might be somewhat reduced, *economic* pasture improvement could not alone effect control on a typical hill farm to-day. At the same time, any increase in the nutritive value of the grazing would undoubtedly improve the condition of the ewe-with-lamb so that she would feed fewer ticks (Milne, 1947*b*). This may be important as sheep are by far the major hosts for *I. ricinus*.

Pasture improvement may have an important place in a limited aspect of the tick problem—that concerned with lowland farms, where ticks are not entirely unknown. The writer knows of one lowland farm in northern England where a field of roughish permanent pasture was infested with ticks by imported hill sheep. The farmer claimed to have got rid of the ticks by ploughing and cropping for several years. Again, certain semi-arable farms in the coastal region of southern Wales have one or two infested fields which are little better than enclosed moorland. Drastic pasture improvement would here be economic and effect tick control. There is no escaping the fact, however, that ticks are pre-eminently a problem of hill farms where pasture improvement cannot by itself effect control economically.

Other measures and tick control

Under present conditions in northern England hill pastures, only a small proportion of ticks is fed by wild fauna. Further, the unfed tick dies in 12 months or less without a host (Milne, 1948*b*). From his experiment (1934), MacLeod (1939) avers: 'Only the most rigorous reduction of available hosts, as, for instance, the complete removal of sheep stock, leaving only the wild fauna, could be relied on to reduce drastically the population level of ticks in a given area. Return of the sheep stock subsequently would result, after a varying period of years, in the increase of ticks to their former level of density.' In his experiment, MacLeod had, by serial dipping over 18 months, killed the major (sheep-supported) fraction of the ticks before excluding the sheep from the pasture. It is doubtful whether the withdrawal of the flock would be effective without such previous dipping. Freed of the grazing pressure of sheep, a hill pasture soon becomes almost knee-deep, favouring enormous multiplication of alternative hosts such as voles, shrews, mice, hedgehogs, rabbits, hares, and all their predators. Apart from rabbits and hares which are kept down, this can be seen in conifer plantings of the Forestry Commission in Northumberland. And in these plantings no diminution in tick population is discernible several years after the exclusion of sheep (unpublished). In any case, for economic and practical reasons, a hill flock cannot be withdrawn from its pasture for any length of time.

In our experiments burnt lime was used to improve the pasture, but it may also have acted initially as an acaricide (cp. Set II trials, 1944 and 1945, Table 1). Application of *fresh* burnt lime might be worth further investigation.

The use of insecticidal sprays and dusts on vegetation has been recorded by Smith & Gouck (1944, 1946), who have reported satisfactory results using D.D.T. on ticks including *I. ricinus scapularis* Say. More research on the effect of such applications on insect life in general is required before recommendations become possible.

As 94–99% of female ticks on a northern England hill pasture are fed by sheep (Milne, 1948*a*), the killing of all females parasitizing the flock would provide a very large measure of control in 3 years. The difficulty is that during the lambing season there must be a lapse of 5–6 weeks between dippings at the time when ticks are at the peak of their activity while, until very recently at any rate, commercial dips have had an effective dipping interval of only 2–3 weeks (Milne, 1945*b*; MacLeod, 1947). But both D.D.T. (Heath, 1946) and Gammexane (Cooper Technical Bureau, 1947) show promise of overcoming this difficulty.

With wise pasture improvement as a minor and not strictly necessary adjunct, tick control on hill-sheep farms is most likely to result from the use of an efficient sheep dip.

Warmest thanks are due to Captain the Hon. Claud Lambton who not only welcomed the trials at Hethpool but also bore the expenses of cultivation for most

of the plots; to his shepherds for their unfailing help; to the Duke's Memorial Fund, Northumberland, for expenses connected with plots N and P; to Messrs F. J. Moralee and R. Stephenson for kindly co-operation enabling study of reseeded sections of their hill farms; to Mr T. P. McNicol, Experimental Officer, for much assistance in field and laboratory; to Mr D. J. Finney, Oxford, for guidance on the statistical side; and to Dr J. MacLeod, Research Fellow of the Veterinary Educational Trust.

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APHIS MIGRATION AND THE EFFICIENCY OF THE TRAPPING METHOD

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(With 5 Text-figures)

Reproducible results were obtained when adhesive traps for aphides were placed at the same height in different parts of a potato field. The total catches in different localities in different seasons can therefore justifiably be compared. During the summer dispersal flights, different species flew at different heights, so traps at one height only are not suitable for comparing the relative abundance of different species. There was less variation in numbers caught on traps at 3–4 ft. above the ground than on traps at 5–6 ft. or at ground-level.

During the first fortnight of July an average of 200 aphides, 1·6% of which were *Myzus persicae*, was caught on traps in the position of potato plants. Most of these were not potato aphides, but it is suggested that some of them might act as vectors of potato viruses. The number of aphides caught was roughly proportional to the volume of free air space around the traps, and as the foliage increased in density the numbers caught on lower traps decreased in proportion.

Wind-speed records within and above the crop are discussed and it is shown that conditions are often suitable for voluntary aphis flight in and near the crop on relatively windy days. Records of catches on the half-traps facing the wind and on the other halves showed that voluntary flight decreased with increasing height.

Brilliant yellow traps, coated with adhesive grease, caught more aphides than white traps, which in turn caught more than black.

The stove-pipe types of sticky aphis traps have been in use for the last 7 years to study aphis migration and its relation to the spread of potato and sugar-beet virus diseases (Broadbent, 1946; Doncaster & Gregory, 1948; Broadbent & Gregory, 1948; Broadbent, Doncaster, Hull & Watson, 1948). Two types of trap have been used, both consisting of lengths of galvanized iron stove-piping, 5 in. in diameter and painted white. The earlier type was 3 ft. long and was coated directly with a grease-banding preparation; the later type was 1 ft. long and the grease was carried on a transparent plastic cover (area 1·3 sq.ft.). Both types of trap have given valuable results in showing the time at which aphides were flying, but the significance of quantitative differences in the catches on traps at different centres or in different seasons was uncertain.

The flight of aphides is largely influenced by wind (Davies, 1936; Thomas & Vevai, 1940), and wind speeds and directions probably vary considerably at different heights near the ground (Geiger, 1927). The experiments described were conducted to determine in what manner the height of the trap above the ground affected the catch, to study wind gradients near the ground and their effect on aphis flight, to determine if the colour of the traps was of significance in attracting aphides,

and if traps placed at the same height in different parts of a potato field gave similar catches.

TRAPPING AT DIFFERENT HEIGHTS IN A POTATO FIELD

Four posts were erected in a crop of Majestic potatoes on Great Harpenden field, Rothamsted; each carried three 1 ft. long traps (Broadbent *et al.* 1948) at heights 2–14 in. (trap C), 32–44 in. (trap B), and 62–74 in. (trap A) above the soil of the potato ridge. The last height is the greatest at which the trap covers can be conveniently changed. The traps C occupied the positions of potato plants in the rows. The traps were all orientated so that the brass strips holding the covers faced north-west; thus, when the covers were examined, they could be divided into four strips which had faced north, west, east and south respectively.

The traps were erected on 19 June 1947 with thirty-four rows of potatoes (80 ft.) between the traps. They were not in operation between 7–10 July when the field was being ridged; they were then re-erected in pairs, 10 rows (24 ft.) apart, with 40 rows (96 ft.) between the two pairs. Another trap was erected, at the same height as the top traps (A) of the other four groups, in an open gangway 24 ft. wide. The covers were changed in the mornings (*c.* 7.00 a.m. G.M.T.) at varying intervals (Table 1). Total numbers of aphides were counted for each quadrant of each trap; after clearing they were examined and the number of *M. persicae* Sulz. per trap was counted (Table 1).

TABLE 1. *Total aphides and* Myzus persicae *trapped at three levels in a potato field*, 1947

			Aphides						*M. persicae*				
			Trap						Trap				
Date	No. of days	Level	1	2	3	4	Total	% of total	1	2	3	4	Total
19–23 June	4	A	16	25	11	12	64	49	0	0	0	0	0
		B	14	11	7	11	43	33	0	0	0	0	0
		C	8	6	5	5	24	18	0	0	0	0	0
		Total	38	42	23	28	131	—	0	0	0	0	0
23–26 June	3	A	47	35	53	51	186	51	0	0	0	0	0
		B	25	22	34	37	118	33	0	0	0	0	0
		C	17	10	19	12	58	16	0	0	0	1	1
		Total	89	67	106	100	362	—	0	0	0	1	1
26 June–7 July	11	A	155	144	185	190	674	35	0	0	1	0	1
		B	145	144	218	164	671	35	1	2	0	0	3
		C	170	80	180	153	583	30	0	0	0	0	0
		Total	470	368	583	507	1928	—	1	2	1	0	4
10–14 July	4	A	164	160	156	152	632	43	2	2	2	0	6
		B	120	136	117	136	509	35	0	0	1	0	1
		C	123	52	53	85	313	22	1	0	0	0	1
		Total	407	348	326	373	1454	—	3	2	3	0	8
14–18 July	4	A	246	218	184	223	871	56	73	69	42	65	249
		B	112	119	126	148	505	32	17	21	19	18	75
		C	64	34	33	49	180	12	4	4	5	3	16
		Total	422	371	343	420	1556	—	94	94	66	86	340

Table 1 (*continued*)

Date	No. of days	Level	Aphides: Trap 1	Trap 2	Trap 3	Trap 4	Total	% of total	*M. persicae*: Trap 1	Trap 2	Trap 3	Trap 4	Total
18–22 July	4	A	—	176	185	—	361	49	—	1	5	—	6
		B	—	214	112	—	326	44	—	1	1	—	2
		C	—	30	26	—	56	7	—	0	1	—	1
		Total	—	420	323	—	743	—	—	2	7	—	9
22–23 July	1	A	—	54	39	—	93	50	—	1	1	—	2
		B	—	43	38	—	81	43	—	1	0	—	1
		C	—	6	8	—	14	7	—	0	0	—	0
		Total	—	103	85	—	188	—	—	2	1	—	3
23–25 July	2	A	79	96	67	74	316	57	0	1	2	0	3
		B	51	64	35	65	215	39	0	0	0	0	0
		C	7	8	4	3	22	4	0	0	0	0	0
		Total	137	168	106	142	553	—	0	1	2	0	3
25–26 July	1	A	67	67	44	61	239	49	1	0	1	3	5
		B	48	63	55	50	216	45	0	0	0	0	0
		C	9	6	5	10	30	6	0	0	0	0	0
		Total	124	136	104	121	485	—	1	0	1	3	5
26–27 July	1	A	66	77	64	40	247	57	1	2	3	0	6
		B	43	36	35	37	151	35	1	1	1	1	4
		C	13	12	4	4	33	8	0	0	0	0	0
		Total	122	125	103	81	431	—	2	3	4	1	10
27–28 July	1	A	47	31	19	30	127	49	8	2	1	5	16
		B	30	20	21	33	104	40	6	4	0	2	12
		C	7	10	1	11	29	11	0	0	0	0	0
		Total	84	61	41	74	260	—	14	6	1	7	28
28–29 July	1	A	76	77	48	52	253	53	5	6	5	2	18
		B	59	74	34	39	206	42	0	2	2	0	4
		C	4	11	5	6	26	5	0	0	1	0	1
		Total	139	162	87	97	485	—	5	8	8	2	23
29 July–1 Aug.	3	A	122	112	87	100	421	48	10	19	7	7	43
		B	101	135	94	79	409	47	4	5	3	4	16
		C	13	9	10	15	47	5	0	1	0	0	1
		Total	236	256	191	194	877	—	14	25	10	11	60
1–4 Aug.	3	A	84	71	71	66	292	53	15	15	10	15	55
		B	49	69	50	58	226	41	8	7	9	7	31
		C	15	8	9	6	38	6	2	1	1	0	4
		Total	148	148	130	130	556	—	25	23	20	22	90
4–8 Aug.	4	A	76	80	83	93	332	52	19	12	23	23	77
		B	60	59	73	58	250	39	12	16	17	11	56
		C	19	9	14	13	55	9	4	0	6	3	13
		Total	155	148	170	164	637	—	35	28	46	37	146
8–14 Aug.	6	A	84	—	—	83	167	67	9	—	—	12	21
		B	33	—	—	38	71	29	7	—	—	5	12
		C	8	—	—	2	10	4	3	—	—	0	3
		Total	125	—	—	123	248	—	19	—	—	17	36

Table 1 (*continued*)

Date	No. of days	Level	Aphides: Trap 1	2	3	4	Total	% of total	*M. persicae*: Trap 1	2	3	4	Total
14–18 Aug.	4	A	23	—	—	25	48	63	1	—	—	1	2
		B	18	—	—	7	25	33	2	—	—	3	5
		C	1	—	—	2	3	4	0	—	—	0	0
		Total	42	—	—	34	76	—	3	—	—	4	7
18–25 Aug.	7	A	38	—	—	23	61	68	1	—	—	0	1
		B	13	—	—	14	27	30	1	—	—	0	1
		C	2	—	—	0	2	2	0	—	—	0	0
		Total	53	—	—	37	90	—	2	—	—	0	2
25 Aug.–1 Sept.	7	A	35	—	—	26	61	64	0	—	—	0	0
		B	15	—	—	12	27	28	0	—	—	0	0
		C	3	—	—	5	8	8	0	—	—	0	0
		Total	53	—	—	43	96	—	0	—	—	0	0
1–8 Sept.	7	A	41	—	—	46	87	57	0	—	—	1	1
		B	36	—	—	18	54	36	1	—	—	0	1
		C	6	—	—	5	11	7	0	—	—	0	0
		Total	83	—	—	69	152	—	1	—	—	1	2
8–15 Sept.	7	A	87	—	—	80	167	67	5	—	—	4	9
		B	39	—	—	34	73	29	1	—	—	2	3
		C	6	—	—	3	9	4	1	—	—	0	1
		Total	132	—	—	117	249	—	7	—	—	6	13
15–22 Sept.	7	A	89	—	—	72	161	67	5	—	—	4	9
		B	27	—	—	32	59	24	1	—	—	2	3
		C	8	—	—	13	21	9	3	—	—	1	4
		Total	124	—	—	117	241	—	9	—	—	7	16
22–29 Sept.	7	A	46	—	—	49	95	58	3	—	—	3	6
		B	35	—	—	25	60	36	0	—	—	0	0
		C	5	—	—	4	9	6	0	—	—	0	0
		Total	86	—	—	78	164	—	3	—	—	3	6
29 Sept.–6 Oct.	7	A	48	—	—	28	76	69	1	—	—	1	2
		B	12	—	—	16	28	25	1	—	—	3	4
		C	3	—	—	4	7	6	0	—	—	1	1
		Total	63	—	—	48	111	—	2	—	—	5	7

The four banks of three traps were operated from 19 June to 8 August, excepting 7–10 and 18–23 July, i.e. a total of 42 days. The total number of aphides caught over that period is given in Table 2, together with the percentage that each catch forms of the total on the three traps on each post. These percentages are very similar for the four traps at each level. Table 3 gives the deviation from the mean of each total catch and these differences expressed as percentages of the mean. In the past, traps of this type have always been used between the limits set by traps A and B, so a better idea of their reliability is obtained by omitting the low traps (C) from consideration. Then the highest deviation from the mean of A + B is less than 5% of the mean.

TABLE 2. *Total aphides trapped 19 June–8 August 1947, with the percentage of the total at each level*

Trap ...	1		2		3		4		Mean	
	No.	%	No.	%	No.	%	No.	%	No.	%
A	1245	49	1193	50	1072	46	1144	47	1164	48
B	857	33	952	39	899	39	915	38	906	37
C	469	18	255	11	342	15	372	15	359	15
Total	2571	—	2400	—	2313	—	2431	—	2429	—

TABLE 3. *Deviations from the mean expressed as numbers and as percentages of the mean*

Trap ...	1		2		3		4		Mean
	No.	%	No.	%	No.	%	No.	%	No.
A	+ 81	+ 7	+ 29	+ 3	− 92	−8	−20	−2	1164
B	− 49	− 5	+ 46	+ 5	− 7	−1	+ 9	+1	906
C	+110	+31	−104	−29	− 17	−5	+13	+4	359
Total	+142	+ 6	− 29	− 1	−116	−5	+ 2	0	2429

Two groups of traps, numbers 1 and 4, were operated until the potatoes were lifted (6 October). Table 4 gives the total catches for these six traps over the whole season.

TABLE 4. *Total aphides on traps 1 and 4, 19 June–6 October, with the percentage of the total at each level*

Trap ...	1		4		Mean	
	No.	%	No.	%	No.	%
A	1736	52	1576	51	1656	52
B	1085	33	1111	36	1098	34
C	511	15	410	13	461	14
Total	3332	—	3097	—	3215	—

The similarity between total catches, over the individual periods and over the whole period, justifies the comparison of catches at different centres so long as the traps are erected at the same height and under similar conditions. It is not often, however, that total catches are compared; interest usually centres on individual species, and especially upon *M. persicae*. Table 5 gives the total catches of *M. persicae*: although the percentage deviation from the mean of traps A + B was between 4 and 14% of the mean, the periodic and total catches on the four traps corresponded with sufficient closeness to enable catches at different centres to be compared, again so long as the traps are operated under standard conditions.

The greatest variation between traps at any one level was between those near the ground (C); the differing conditions of shelter and of wind eddies at this level must have profoundly affected the catch. The catches on the middle traps (B) were more

comparable than were those on the upper traps (A), for total aphides (1–5% deviation in B, 2–8% in A) and for *M. persicae* (2–16, 2–20%).

TABLE 5. *Total* Myzus persicae *trapped, with the percentage of the total at each level*

Trap ...	1		2		3		4		Mean	
	No.	%	No.	%	No.	%	No.	%	No.	%
					19 June–8 August					
A	134	69	127	67	96	60	122	71	120	67
B	49	25	58	30	52	32	43	25	51	28
C	11	6	6	3	13	8	7	4	9	5
Total	194	—	191	—	161	—	172	—	180	—
					19 June–6 October					
A	159	66	—	—	—	—	148	69	154	67
B	63	26	—	—	—	—	50	23	57	25
C	18	8	—	—	—	—	17	8	18	8
Total	240	—	—	—	—	—	215	—	229	—

TABLE 6. *Total aphides and* Myzus persicae *trapped at level A; traps 1–4 over potatoes, trap 5 over adjacent bare ground*

	Total aphides					*M. persicae*				
Date Trap ...	1	2	3	4	5	1	2	3	4	5
19–23 June	16	25	11	12	17	0	0	0	0	0
23–26 June	47	35	53	51	28	0	0	0	0	0
26 June–7 July	155	144	185	190	233	0	0	1	0	1
10–14 July	164	160	156	152	157	2	2	2	0	1
14–18 July	246	218	184	223	184	73	69	42	65	59
Total	628	582	589	628	619	75	71	45	65	61

Table 6 gives the catches from trap 5 from 19 June to 18 July, compared with the four A traps above the crop. It is clear that, with traps at the height normally used, similar catches of total aphides or of *M. persicae* are obtained whether the trap is above the crop or above adjacent bare ground.

DISTRIBUTION OF SPECIES AT DIFFERENT HEIGHTS

The catches over 19 trapping days between 26 June and 18 July have been examined in greater detail and the numbers of the predominant species counted. The three commonest species were *Metopolophium dirhodum* (Walk.), *Cavariella aegopodii* (Scop.) and *Myzus persicae*. Black aphides were present in large numbers, but because of the difficulty of identifying these with any certainty, they have been grouped together and called '*Aphis fabae* complex'. The three species plus the '*A. fabae* complex' comprised 68% of the total catch on the upper traps (A), 75% on the middle (B), 81% on the lower (C), and 73% of the total catch on all traps.

In order to compare species, the numbers have been converted to percentages of the total at the three levels for each species (Fig. 3). It is obvious that during this period the four species tended to fly at different levels. *Myzus persicae* and *Metopolophium dirhodum* were more numerous on the upper traps, *Cavariella aegopodii* on the lower traps, at least during the peak period of flight for this species (26 June–7 July). From the catches on the A traps it would seem that, over the period under consideration *Myzus persicae* (256) and *Cavariella aegopodii* (229) were present in approximately equal numbers, whereas on all the traps at the different levels *C. aegopodii* (827) was more than twice as numerous as *Myzus persicae* (352). Thus traps situated at the height of 6 ft. cannot be used to compare the relative abundance of different species. In studying the spread of virus disease in a field, aphides in the air near the crop are of particular interest, and it is important to note that the middle traps (B) gave the most consistent results, since the percentages of each species and of total aphides occurring on these traps varied less than those either on upper or lower traps. It has already been shown that there was less variation in total numbers on the middle traps than on the others. In general, it appears that the best comparison between different centres or between species would be obtained with the traps at heights of 3–4 ft., although at this height fewer numbers are caught of certain species, including *M. persicae*. This would be a disadvantage in years when the species is not abundant, but it might be overcome by making the traps more attractive and having more than one at each centre.

Virus transmission in the field

The relatively large number of aphides of different species caught on the traps at ground level in the position of potato plants raises some interesting problems. Over the fifteen trapping days between 26 June and 14 July an average of 224 aphides per trap was caught on the C traps (Table 1). Thus it may be assumed that about 200 aphides, on the average, visited each plant during the first fortnight in July. A large number of these would test the plants by inserting their stylets, but would not necessarily stay to feed (Moericke, 1941). The testing action might be sufficient for transmission of potato virus *Y* by vector species and this may account for the large spread of the disease found in many years at different centres when potatoes are sampled in the second half of July (Doncaster & Gregory, 1948; Broadbent & Gregory, 1948), transmission which must have taken place before the potato aphides had built up large populations on the plants. It has been assumed that most of this early spread is caused by alate *M. persicae*, together with *Macrosiphum solanifolii* and *Aphis rhamni*, but other species which do not regularly breed on potatoes have been shown capable of spreading potato viruses (Whitehead, 1930, 1931; Smith, 1931; Loughnane, 1939; Bawden & Kassanis, 1947), and many common species have not been tested. Though most of the vectors, other than the potato aphides, were relatively ineffective, the large numbers present may add to the total of transmission.

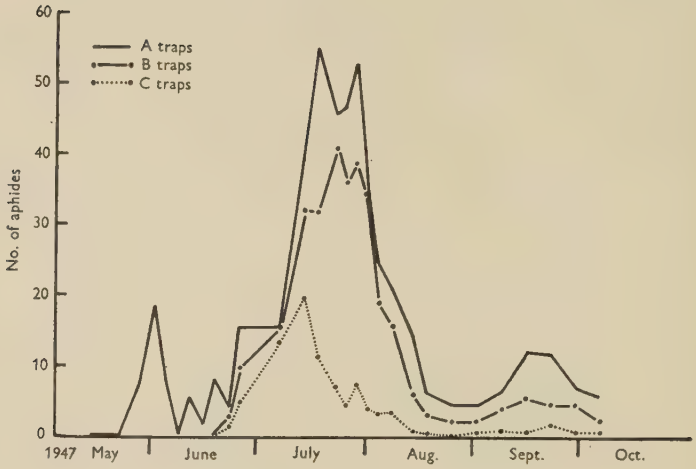

Fig. 1. Average total aphides per trap per day.

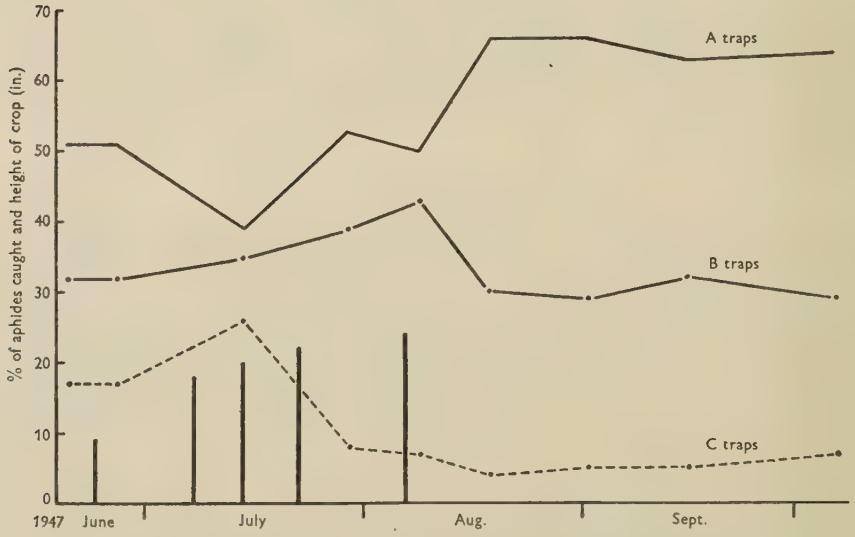

Fig. 2. Percentage of the total catch of aphides formed by the catch at each level. The vertical lines indicate the height of the crop.

HEIGHT OF CROP AND ITS EFFECT ON HEIGHT OF APHIS FLIGHT

Fig. 1 shows the average catch per day at each level. Fig. 2 shows the percentage of the total catch formed by the catch at each level, at approximately fortnightly intervals. Total numbers on the upper and middle traps reached a peak in the last fortnight of July, at the time of the summer dispersal, after which there was a rapid decline until the autumn dispersal in September, when there was a small increase.

The peak on the lower traps was a few days earlier than on the others, during the second week of July. The catches on trap 5 during May and June are also given, and indicate that the spring migration occurred in late May. The catch consisted of many species, with *Cavariella aegopodii* (19% of total) and *Phorodon humuli* (Schr.) (17%) predominating.

During the first week of July the crop was 16–18 in. high and the plants were not meeting in the rows. At this time large numbers of aphides were caught, in the proportions at the three levels of A, 35%, B, 35%, C 30%. As the crop grew and met in the rows, the proportions changed, and by the first week in August, when the haulm was dense and 20–28 in. high, were about A, 50%, B, 45%, C, 5%. By the middle of August the proportions were about A, 65%, B, 30%, C, 5%, at

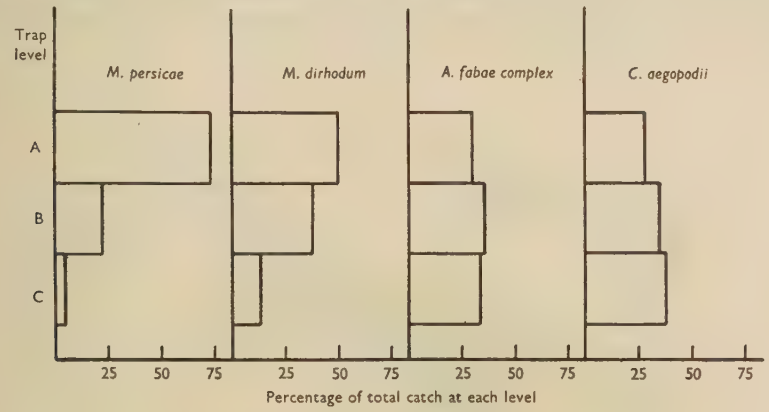

Fig. 3. Catches of the four dominant species over the peak migration period (26 June–18 July 1947).

which they remained until the end of the season, even after the haulms collapsed at maturity.

On 18 July the crop was about 24 in. high, i.e. it was about 8 in. below the B traps and 10 in. above the C traps. The plants were not touching between rows, which ran from 30° east of north to 30° west of south. There follows an estimate of the volume of free-air space between the plants around the traps, the numbers of aphides caught over periods during which the measurements were made, and the number of aphides per 1000 cu.in. of space:

Traps ...	1C	2C	3C	4C
18 July 1947:				
Volume (cu.in.)	22,600	10,900	9,350	14,800
Aphides trapped 14–22 July	128	64	59	95
Aphides per 1000 cu.in. of space	5·7	5·9	6·3	6·4
1 Aug. 1947:				
Volume (cu.in.)	16,000	11,600	6,900	8,400
Aphides trapped 29 July–4 Aug.	32	28	24	27
Aphides per 1000 cu.in. of space	2·0	2·4	3·5	3·2

It is clear that as the foliage increased in density the number of aphides caught on the lower traps decreased (see also Fig. 2). The number of aphides caught was roughly proportional to the volume of free-air space around the trap, from which it may be assumed that more aphides would visit each plant in an open crop than in a close-planted one. A similar observation was made by van der Plank & Anderssen (1944) regarding thrips on tobacco.

WIND-SPEED RECORDS

Records were made on many occasions of wind speed at 1 and 2 m. above the soil with two sensitive cup anemometers. A number of readings with one of these anemometers in the crop, compared with the other at 2 m., was also made, in addition to direct readings with a hot-wire anemometer. Fig. 4 gives the wind

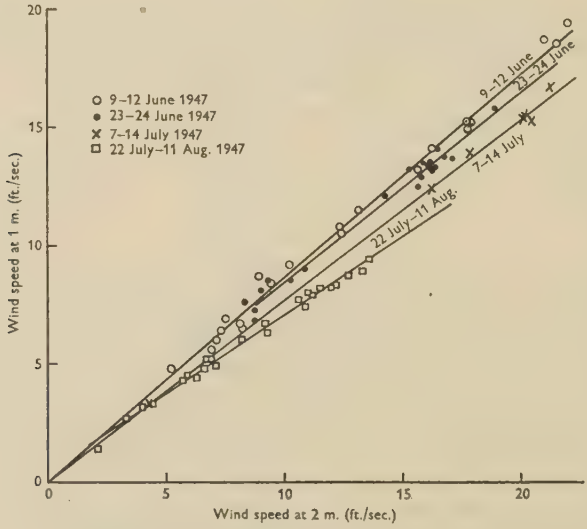

Fig. 4. Regression lines showing correlation between wind at 2 m. and at 1 m. above the ground.

speed at 2 m. plotted against that at 1 m. The relation between these two levels is linear, and the slope of the regression line varies as the crop increases in height. In Fig. 5 the wind speeds in the crop and at 10 in. above the ground before the crop was fully through, are plotted against wind speed at 2 m. In this case there is a wide scatter of points since the anemometer was placed in varying positions in the crop at different times and experienced different degrees of shelter. Davies (1936) showed in laboratory experiments that *Myzus persicae* ceased voluntary flight at wind speeds over 3·75 m.p.h. This fact has often been discussed in relation to aphis migration (Davies, 1939; Thomas & Vevai, 1940; Loughnane, 1940; Greenslade, 1941;

Profft, 1939; Heinze & Profft, 1940; Hansen, 1941; Broadbent, 1946; Doncaster & Gregory, 1948) and it has generally been agreed that aphides will only undertake voluntary flight at wind speeds under 4–5 m.p.h. But the records of wind speeds of 4 m.p.h., used in these discussions, have usually been made at recognized meteorological stations, at heights of 10 m., or have been based on the Beaufort Scale. On the average the wind speed at 2 m. is 0·78 that at 10 m. (*Meteorological Observers' Handbook*, H.M.S.O.), so that when the wind speed is 4 m.p.h. (5·9 ft./sec.)

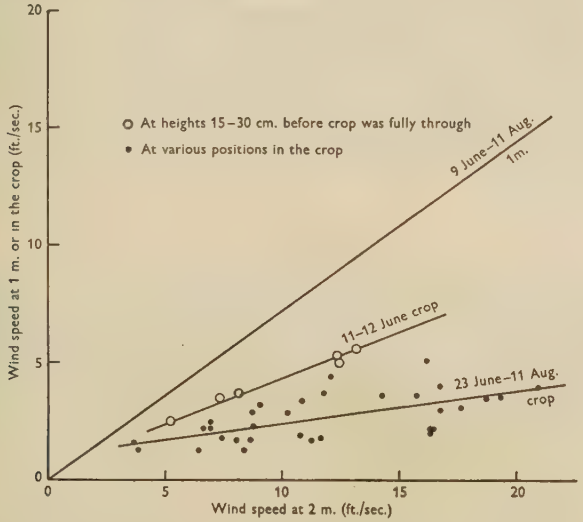
Wind speed at 1 m. or in the crop (ft./sec.)
Wind speed at 2 m. (ft./sec.)
0
5
10
15
20
O At heights 15–30 cm. before crop was fully through
• At various positions in the crop
9 June–11 Aug. 1 m.
11–12 June crop
23 June–11 Aug. crop

Fig. 5. Regression lines showing correlation between wind speeds at 2 m. and at 1 m. (mean from Fig. 4), and in the potato crop.

TABLE 7. *Comparative wind speeds at different heights*

	In the crop		1 m.		2 m.		10 m.	
Height	m.p.h.	ft./sec.	m.p.h.	ft./sec.	m.p.h.	ft./sec.	m.p.h.	ft./sec.
Very young	4·0	5·9	—	—	9·6	14·1	12·3	18·1
crop (June)	—	—	4·0	5·9	4·7	6·9	6·0	8·8
Dense haulm	4·0	5·9	—	—	24·7	36·2	31·6	46·4
(July–Aug.)	—	—	4·0	5·9	5·7	8·3	7·3	10·7
General	—	—	—	—	4·0	5·9	5·1	7·6

at 2 m. it will be 5·1 m.p.h. (7·6 ft./sec.) at 10 m., although the vertical gradient of wind speed is in general smaller with light winds and by day than with strong winds and by night (Giblett, 1932). When the crop has grown the ratio will be slightly different, because the effective height of the upper anemometer will be something less than 2 m. The comparative wind speeds at different heights have been read from Figs. 4 and 5 and compiled in Table 7. It will be seen that in the early part

of the season, when the plants are just coming up and are first visited by aphides, an average wind speed of 4 m.p.h. at 10 in. above the ground would require an average of 12·3 m.p.h. at 10 m. Later in the season an average wind speed of 4 m.p.h. just above the crop (at 1 m. above the soil) would necessitate an average of 7·3 m.p.h. at 10 m., and an average of 4 m.p.h. within the crop would require an average of about 32 m.p.h. at 10 m. Direct readings with the hot-wire anemometer showed that the wind speed in the crop fluctuated greatly and gusts of over 4 m.p.h. often occurred when the average wind speed at 2 m. was 8 m.p.h. However, it is clear that much voluntary aphis movement can take place among and just above the plants even on windy days (Broadbent, 1946). Also many aphides flying near the crop will be caught up in vertical wind currents and may be transported long distances involuntarily, as was pointed out by Profft (1939). The number of occasions when conditions in the crop are suitable for take-off will decrease as the wind speed increases, and this no doubt largely accounts for the occurrence of fewer aphides in the air on windy days.

DISTRIBUTION OF APHIDES ON THE TRAP SURFACES

The total numbers of aphides caught on the four sectors of traps at three heights, over the periods 25–29 July, when the trap covers were changed daily, and 4–8 August, are given in Table 8. The wind was relatively steady in direction on these

TABLE 8. *Distribution of aphides over trap surfaces; totals for four traps*

			Trap A				Trap B				Trap C			
Date	Wind direction	m.p.h.	N.	E.	S.	W.	N.	E.	S.	W.	N.	E.	S.	W.
25–26 July	S.W.–N.W.	0–5	40	30	77	92	25	50	72	69	4	10	6	10
26–27 July	W.	5–10	20	39	78	110	19	27	47	58	5	17	8	3
27–28 July	E.–S.E.	5–10	19	71	29	8	17	48	25	14	5	6	11	7
28–29 July	Varied	calm	52	55	76	70	39	43	67	57	2	8	8	8
4–8 Aug.	S.W.–N.W.	5–10+	54	11	78	189	36	9	64	141	15	14	18	8

Totals for the half-traps facing the prevailing wind and for the other half

		Trap A		Trap B		Trap C	
Date	Quadrants facing wind	Facing wind	Against wind	Facing wind	Against wind	Facing wind	Against wind
25–26 July	W.+½ (N.+S.)	151	88	118	98	15	15
26–27 July	W.+½ (N.+S.)	159	88	91	60	10	23
27–28 July	E.+S.	100	27	73	31	17	12
4–8 Sept.	W.+½ (N.+S.)	255	77	191	59	25	30
	Totals	665	280	473	248	67	80
Percentage of total on each trap		70	30	66	34	46	54

days. The catches have been tabulated, giving the catches on the half-traps facing the prevailing wind and on the other halves. On the calm day 28–29 July, the catches were well distributed around the traps, and this day has therefore been omitted from the second part of the table.

On the average, the half facing the wind received 70% of the catch on the A traps, 66% on the B, and 46% on the C. At this time of the year the wind speed at 2 m. relative to that at 1 m. was 1·45:1 (Fig. 4). The total catches on the half-traps facing the wind over the 7 days were: A:B=665:473, i.e. 1·41:1. It might be inferred that these aphides were deposited at rates proportional to the wind speed, and that, therefore, the insects were randomly distributed throughout this layer of air. In Table 4 are given the catches from 19 June to 6 October, which for A and B traps are in the ratio 1·28:1. From Fig. 4 the wind speeds over this period were in a similar ratio. On the other hand, Table 1 shows that, over short periods, the number of aphides caught was often not proportional to wind speed. It is hoped that a study of the air flow around the trap and of the deposition of aphides on it in a wind tunnel will give more information on this subject.

The shelter and wind eddies operating around the traps at ground level evidently prevented the catches from bearing any direct relation to wind speed or direction.

Attraction of colour

When trapping, it is important to know if the number of any species caught on a given surface area in a given time represents the average number contained in the volume of air which had flowed past the trap in that time, or if the number is greater owing to attraction exerted by the trap, or less as a result of repulsion. The normal colour of the sticky trap was old gold (Ridgway, 1912), the colour of the coating of grease over the white paint. This colour was tested against black and primuline yellow (Ridgway, 1912) in two experiments.

In the first experiment, three 1 ft. length traps were fastened one above the other so that the top of the upper trap was 6 ft. from the ground. They were divided into four quadrants facing north, west, south and east, and each quadrant was divided into three horizontally. The resulting 4-in. squares were painted black, white and yellow, so that the three colours occurred in each quadrant. The squares were arranged so that no two of the same colour were adjacent. The plastic covers of the traps were marked into squares so that when they were removed the catch in each square could be recorded according to direction and colour.

This trap was erected near allotments at Rothamsted and cleared at weekly intervals over a period of 5 weeks. Over the whole period equal numbers of aphides were trapped on the white and black squares, whereas 1·23 times as many were caught on the yellow squares. In the first and last weeks, 11 of the 14 days were relatively calm (with average day winds under 5 m.p.h.), the wind in the second week was variable, while the third and fourth were windy (11 days with winds averaging over 5 m.p.h.). The catches showed the following ratios:

	Black	White	Yellow
Weeks 1 and 5 (calm)	1·00	0·86	1·54
Weeks 3 and 4 (windy)	1·00	1·02	1·16

Attraction would presumably be greater on calm days, when the aphides could direct their flight, and from the above figures it appeared that the aphides were attracted to the yellow squares.

The numbers of *M. persicae* caught during the 5-week period were:

	Black	White	Yellow
No.	12	14	20
Ratio	1·00	1·17	1·67

Whereas these figures showed a tendency for the aphides to be attracted to yellow, the attraction was not marked, perhaps because of the small size of the squares. Only in the very calmest weather could a trap of this kind give satisfactory results; at other times the aphides would probably be blown on to the trap at random. Another experiment was therefore carried out.

In the second experiment, three 1 ft. long traps were erected separately near the multicoloured trap, so that the top of each trap was 6 ft. from the ground. One trap was white, one black and one yellow. They were operated for a period of 40 days, their position relative to one another being changed twice, so that each occupied a given position for about one-third of the period. For 20 days the trap covers were changed daily. There was apparently no effect produced by the position of the trap. The aphides caught over the period were:

		Black	White	Yellow
Total aphides	No.	347	428	529
	Ratio	1·00	1·23	1·52
M. persicae	No.	27	33	40
	Ratio	1·00	1·22	1·48

An analysis of variance on total daily catches gave the following results:

Daily mean difference between:

Black and white traps	$2{\cdot}025 \pm 1{\cdot}109$
White and yellow traps	$2{\cdot}525 \pm 1{\cdot}318$
Black and yellow traps	$4{\cdot}550 \pm 1{\cdot}236$

Thus there was a suggestion of white being more attractive than black, and of yellow more than white, yet these differences were barely significant. Yellow, however, was significantly more attractive than black. The attraction may be greater than the differences between catches suggest because of the wind rendering choice of direction on the part of the aphides difficult, i.e. on windy days aphides would be deposited indiscriminately on the three traps. However, the ratios between catches on the three traps on days when they were changed daily were:

	Black	White	Yellow
On 13 windy days (wind over 5 m.p.h.)	1·00	1·23	1·42
On 7 calm days (wind under 5 m.p.h.)	1·00	1·25	1·41

This would suggest that wind speed had little effect on attraction. On the other

hand, if the daily catch is divided into two parts (1) that on the half-trap facing the prevailing wind, and (2) that on the other half, then the following totals are obtained:

	Facing wind			Against wind		
	Black	White	Yellow	Black	White	Yellow
No.	184	220	231	49	78	110
Ratio	1·00	1·20	1·26	1·00	1·59	2·24

Thus the catch on the half-trap facing the wind was 3·8 times that on the other half on the black trap, 2·8 times on the white and 2·1 times on the yellow. If it is assumed that most of the aphides on the side away from the wind had flown on to the trap voluntarily, then white attracted 59% more than black, and yellow 65% more than white.

It may be that black repels, rather than yellow attracts, but assuming the latter, and assuming the catch on the black trap to be due entirely to deposition by wind and that such involuntary deposition was the same for all three traps, then the numbers attracted to white and yellow traps would be:

	White	Yellow	Ratio W.:Y.
Facing wind	36	47	1·00 : 1·31
Against wind	29	61	1·00 : 2·10

In sum it appears that yellow attracts aphides, and that the brighter the colour, the greater the number attracted.

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EXPERIMENTS ON THE SPREAD OF RUGOSE MOSAIC AND LEAF ROLL IN POTATO CROPS IN 1946

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(With 5 Text-figures)

A series of experiments on the spread of potato rugose mosaic (virus *Y*), and leaf roll, which has been in progress on a uniform plan since 1943, was ended in 1946. Mean values for thirteen centres in England and Wales showed that in 1946 69% of the infections with virus *Y* and 48% of those with leaf-roll virus reached the tubers of Majestic potatoes by the beginning of August. There was usually little subsequent increase of rugose mosaic, but a late increase of leaf roll was associated with a relatively high initial spread. Three-quarters of the virus *Y* and over half the leaf-roll infections occurred within five plants distance of the source. There was no close correlation between the spread of either virus and the maximum number of *Myzus persicae*, either apterous forms on the plants or alate forms caught on adhesive traps, but centres with high trap catches in July and August showed pronounced late season spread of leaf roll. There were marked differences at different centres in the relative spread of the two viruses. The amount of spread and the gradients from source of infection of the two viruses are compared over the period 1943–6.

Since 1943 a series of co-operative field experiments on the spread of potato rugose mosaic caused by potato virus *Y* and leaf roll has been in progress in various parts of the country. The chief object of these experiments has been to obtain information on the relative amounts of transmission of the two viruses at various distances from a standardized source of infection at different times during the growing period and in different localities and seasons. Results for the years 1943–5 have already been described by Doncaster & Gregory (1948). The series ended during 1946 with experiments at thirteen centres listed in Table 1.

The design of the plot used in 1946 is shown in Fig. 1. The plot of 28 rows × 34 plants was planted with Scottish Stock Seed Majestic potatoes from Aberdeenshire, with a double row of alternating rugose mosaic and leaf-roll infected tubers planted across the middle of the rows. Samples were taken at fortnightly intervals from the middle of July to the end of September. On the first sampling date one tuber was removed from each of the thirty test plants in each of the eight rows: 1, 4, 7, 10, 13, 16, 19, 22. The tubers were taken from one side only of the rows and were collected in six bags corresponding to the six sections A–F on the plan. On the second sampling date rows 2, 5, 8, 11, 14, 17, 20, 23 were sampled, and on the third occasion rows 3, 6, 9, 12, 15, 18, 21 and 24. For the fourth, fifth and sixth sampling occasions tubers were removed from the same rows as for the first, second and third sampling respectively, but from the side of the row opposite to that from

which they were removed earlier; this was done to avoid taking any tubers that might have become detached from their stolons during the first sampling. The tubers were stored at the University College of Nottingham School of Agriculture, Sutton Bonington, planted in the first week in May 1947 and examined for rugose mosaic and leaf roll in the last week in June (Tables 1 and 2).

The planting design in 1946 was the same as in previous years, but the sampling method was modified so that the number of tubers in each sample was doubled, and the plot was divided into eight blocks, each of which was sampled on each of the six fortnightly sampling occasions.

The infector tubers were saved from typical rugose mosaic and leaf-roll Majestic plants growing at Sutton Bonington during 1945. The plots at the various centres were planted as early as convenient locally. All were well manured, four of the thirteen had artificials only (Bretton, Cardiff, East Malling and Reading), the rest had normal to heavy dressings of farmyard manure in addition to artificials.

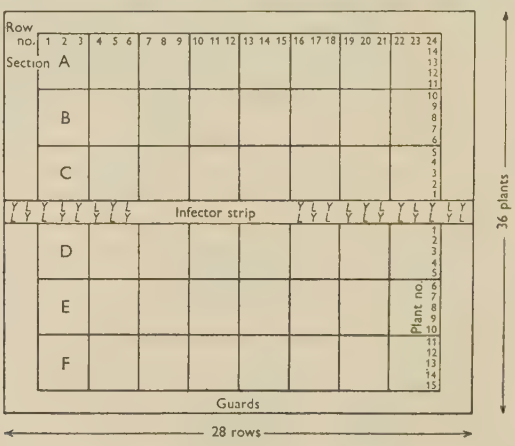

Fig. 1. Standard design of plot as used at all centres in 1946.

SPREAD OF DISEASE IN 1946

The incidence of both leaf roll and rugose mosaic was found to increase during the season, but as in previous years a large amount of spread occurred in the early part of the season. The mean values for all centres (Tables 1 and 2) show that of the total spread during the season 69% of the infections with virus *Y* and 48% of those with leaf roll had already reached the tubers by the second sampling occasion (beginning of August).

A decline in the percentage of infected tubers taken at the final sampling date was again noted at some centres, in spite of alterations made in the method of sampling during 1946 in order to eliminate any spurious effect due to asymmetry of the plot. The fall is shown with rugose mosaic at Baston Fen, Cardiff and Reading; and with

leaf roll at Bretton, Cardiff, East Malling, Harper Adams, Postland, Seale-Hayne and Slough. The effect is unexplained.

The means for all centres give the impression of a continuous increase of disease, but data from individual centres show that the increase of a virus is occasionally

TABLE 1. *Percentage rugose mosaic according to sampling occasion and position* 1946 (*from sample tubers scored in* 1947)

	Occasion							Position					
Centre	1	2	3	4	5	6	Mean	A	B	C	D	E	F
Askham Bryan	5·0	5·5	9·2	6·6	8·6	10·5	7·6	2·4	4·0	17·6	15·9	2·7	2·2
Baston Fen	2·6	4·2	4·8	6·4	5·3	—	4·7	1·2	1·7	7·0	11·4	4·5	1·6
Bretton	—	1·3	0·0	1·5	0·0	0·5	0·6	0·0	0·0	1·9	0·0	1·3	0·6
Cardiff	8·2	10·8	13·5	13·5	15·7	10·2	12·0	5·0	9·1	30·0	24·1	3·6	0·0
East Malling	0·4	0·5	2·2	0·4	1·4	1·9	1·1	1·4	0·0	1·8	1·8	0·4	1·4
Goole	4·6	1·3	4·3	1·8	3·5	3·1	3·1	1·7	2·2	6·9	5·6	1·4	0·9
Harper Adams	6·1	9·9	10·6	12·6	10·8	12·5	10·4	1·0	1·9	20·1	25·1	8·8	4·2
Postland	1·1	1·8	2·6	2·6	3·5	10·0	3·6	0·0	1·5	8·1	7·4	2·3	0·0
Reading	5·8	4·3	8·0	10·3	6·8	5·5	6·8	2·3	8·7	13·4	17·6	3·6	2·7
Rothamsted	7·6	6·9	6·1	9·4	8·5	11·4	8·3	4·5	5·5	19·9	15·8	1·9	2·0
Seale-Hayne	1·4	2·8	1·4	2·6	2·0	3·2	2·2	0·5	2·0	4·8	5·7	0·5	0·0
Slough	3·5	1·3	2·5	5·6	5·2	4·9	3·8	1·3	2·8	6·9	6·7	1·7	4·7
Sutton Bonington	7·0	12·3	7·8	12·3	15·5	15·0	11·6	4·1	4·1	22·7	28·4	5·7	4·0
Mean	4·4	4·8	5·6	6·6	6·7	7·4	—	2·0	3·3	12·4	12·7	3·0	1·9

TABLE 2. *Percentage leaf roll according to sampling occasion and position* 1946 (*from sample tubers scored in* 1947)

	Occasion							Position					
Centre	1	2	3	4	5	6	Mean	A	B	C	D	E	F
Askham Bryan	5·0	9·2	9·2	10·9	18·0	18·9	11·9	13·7	12·9	19·5	21·6	1·3	2·2
Baston Fen	1·5	12·5	20·0	26·6	29·0	—	17·9	10·8	11·6	30·8	33·6	12·3	9·4
Bretton	—	1·3	1·4	4·0	8·3	3·7	3·7	1·3	4·2	5·1	6·4	4·5	1·3
Cardiff	4·1	6·1	5·2	7·0	9·2	5·4	6·2	2·3	7·0	12·1	9·7	3·6	2·2
East Malling	10·2	11·2	14·9	16·1	15·1	14·8	13·7	5·8	6·2	33·2	24·9	7·2	5·5
Goole	5·7	3·5	6·9	12·5	12·7	11·2	8·7	3·9	10·0	12·4	11·3	6·3	8·5
Harper Adams	4·2	6·1	5·3	9·7	11·7	7·3	7·4	1·6	5·7	11·4	14·7	8·8	1·9
Postland	3·2	10·4	24·3	31·6	52·0	47·0	28·1	25·3	21·1	45·0	32·4	22·3	11·7
Reading	5·3	2·4	4·9	7·3	4·5	7·2	5·3	7·3	17·5	4·3	10·5	5·0	3·1
Rothamsted	10·9	14·2	15·1	15·7	23·1	22·4	16·9	18·6	11·3	30·0	28·8	10·6	3·0
Seale-Hayne	1·4	1·7	1·9	2·1	3·4	1·8	2·1	1·5	0·5	5·9	3·8	0·5	0·5
Slough	4·4	7·4	2·5	8·4	7·9	5·8	6·1	3·0	1·4	8·3	10·5	6·0	7·7
Sutton Bonington	3·1	9·5	13·7	14·1	19·6	22·6	13·7	1·8	4·1	32·3	31·1	6·7	5·4
Means	4·9	7·3	9·6	12·8	16·5	14·0	—	7·5	8·7	19·3	18·4	7·3	4·8

discontinuous in time. At any one date one or both viruses may be transmitted (compare Askham Bryan and Baston Fen). On the other hand, the increase may be continuous throughout the season as at Postland.

The linear regression of percentage diseased plants on time of sampling has been calculated for each crop and is shown in Table 3 for comparison with data from previous years (Doncaster & Gregory 1948, Appendix G). The estimated percentage

on 7 August is given in the table and the increment for a subsequent period of 28 days. (Apparent decreases were found with rugose mosaic at Bretton and Goole but these were both small.) The data, smoothed as regressions, show the usual features noted in previous years: (1) relatively large proportion of both diseases present by 7 August; (2) very little subsequent increase of rugose mosaic; (3) late increase of leaf roll associated with relatively high initial value.

The gradients of infection, shown by the relative percentage infections in positions A–F, were of the usual order, and showed a high proportion of infections in the first five plants (74% of the rugose mosaic and 58% of the leaf roll). Some anomalous high values with leaf roll in position A were found at Rothamsted and Postland, both probably coming from the virus infection in the surrounding stocks.

TABLE 3. *Summary of* Myzus persicae *infestation and increase of rugose mosaic and leaf roll (calculated from linear regressions)*

(Compare Doncaster & Gregory, 1948, Appendix G)

	M. persicae			Mean infections per infector unit*		Rugose mosaic (%)		Leaf roll (%)	
Centre	Max. per 100 leaves	Date of max.	Total on trap	Rugose mosaic	Leaf roll	7 Aug.	+28 days	7 Aug.	+28 days
Askham Bryan	76	22 July	10	1·7	2·0	6·7	2·0	9·2	5·6
Baston Fen	414	29 July	64	0·9	3·2	3·2	1·5	7·9	13·8
Bretton	50	26 Aug.	9	0·1	0·6	0·8	0·3	1·9	2·3
Cardiff	8	30 July	6	2·7	1·1	11·3	1·4	5·7	1·0
East Malling	62	8 July	11	0·2	2·8	0·9	0·5	12·6	2·0
Goole	38	13 Aug.	—	0·6	1·2	3·0	2·0	7·2	3·5
Harper Adams	66 (?)	2 Aug. (?)	7	2·3	1·3	10·4	0·05	7·0	0·6
Postland	250	29 July	62	0·8	3·9	2·2	2·8	11·8	20·0
Reading (Shinfield)	4 (?)	19 July (?)	—	1·6	0·7	6·8	0·1	5·0	0·8
Rothamsted	77	11 July	5	1·8	3·0	7·7	1·6	14·5	4·8
Seale-Hayne	44 (?)	4 July (?)	9	0·5	0·5	2·0	0·4	1·9	0·4
Slough	200	13 June	18	0·7	0·9	3·4	1·2	5·7	0·8
Sutton Bonington	952	18 July	78	2·6	3·1	10·2	3·1	11·1	5·6

* Calculated from Sections C and D only.

OBSERVATIONS ON APHIDES

Counts of aphides per 100 leaves were made at intervals during the growing season and are given in Table 4. The method of counting was to take one upper, one middle and one lower leaf from thirty-three plants chosen at random in the plot. *Aphis rhamni* Fonsc., *Macrosiphum solanifolii* (Ashm.) and *Aulacorthum solani* (Kalt.) were not common at most centres and *Myzus persicae* (Sulz.) only is shown graphically in Figs. 2 and 3. The months were divided into four periods: 1–8, 9–16, 17–24, 25–31, and all data were fitted into these periods.

At some centres the number of aphides per plant was estimated by assessing the average number of leaves per plant and multiplying by 1/100 of the aphis count.

TABLE 4. *Potato aphides per 100 leaves**

Peak figures for *Myzus persicae* in italic

Centre		June				July				August				September		
		8	16	24	30	8	16	24	31	8	16	24	31	8	16	24
Askham Bryan	*P*	—	0	0	10	18	—	*76*	30	—	8	4	—	14	2	0
	R	—	0	0	0	0	—	0	0	—	0	0	—	0	0	0
	S	—	0	3	17	63	—	94	80	—	2	14	—	2	0	0
Baston	*P*	3	—	1	—	80	248	—	*414*	—	160	—	7	—	—	0
	R	0	—	3	—	9	53	—	57	—	92	—	2	—	—	0
	S	0	—	0	—	0	6	—	16	—	24	—	0	—	—	0
Bretton	*P*	—	—	—	—	0	—	10	10	—	24	—	*50*	—	—	32
	R	—	—	—	—	0	—	0	0	—	1	—	2	—	—	0
	S	—	—	—	—	1	—	1	0	—	0	—	2	—	—	1
Cardiff	*P*	—	—	4	—	6	—	6	*8*	—	—	—	—	—	—	—
	R	—	—	0	—	0	—	41	14	—	—	—	—	—	—	—
	S	—	—	20	—	48	—	0	2	—	—	—	—	—	—	—
East Malling	*P*	—	—	4	—	*62*	—	8	—	5	—	3	—	32	—	—
	R	—	—	0	—	3	—	0	—	0	—	0	—	4	—	—
	S	—	—	1	—	45	—	40	—	16	—	2	—	10	—	—
Goole	*P*	—	—	2	—	12	—	26	—	—	*38*	4	—	0	—	—
	R	—	—	0	—	0	—	0	—	—	0	0	—	0	—	—
	S	—	—	0	—	8	—	32	—	—	2	1	—	0	—	—
Harper Adams	*P*	—	—	—	—	5	—	—	—	*66*	—	—	—	3	—	—
	R	—	—	—	—	0	—	—	—	0	—	—	—	0	—	—
	S	—	—	—	—	3	—	—	—	37	—	—	—	1	—	—
Postland	*P*	0	—	12	—	51	109	—	*250*	—	35	—	5	—	—	0
	R	0	—	0	—	0	0	—	1	—	3	—	0	—	—	0
	S	0	—	0	—	0	4	—	46	—	10	—	1	—	—	0
Reading	*P*	—	—	—	—	—	—	4	0	—	—	—	—	—	—	—
	R	—	—	—	—	—	—	3	4	—	—	—	—	—	—	—
	S	—	—	—	—	—	—	3	0	—	—	—	—	—	—	—
Rothamsted	*P*	11	10	—	34	—	*77*	—	13	—	14	—	16	—	—	0
	R	5	1	—	15	—	13	—	5	—	4	—	13	—	—	0
	S	4	0	—	0	—	16	—	4	—	3	—	1	—	—	0
Seale-Hayne	*P*	—	—	—	—	*44*	—	8	—	8	—	—	—	—	—	—
	R	—	—	—	—	0	—	1	—	0	—	—	—	—	—	—
	S	—	—	—	—	10	—	0	—	2	—	—	—	—	—	—
Slough	*P*	—	*200*	—	134	—	29	—	17	0	—	—	—	—	—	—
	R	—	16	—	17	—	8	—	13	0	—	—	—	—	—	—
	S	—	27	—	34	—	5	—	0	20	—	—	—	—	—	—
Sutton Bonington	*P*	—	—	9	—	47	—	*952*	246	—	—	7	6	—	—	—
	R	—	—	0	—	1	—	34	14	—	—	1	1	—	—	—
	S	—	—	0	—	10	—	108	66	—	—	1	1	—	—	—

* *Aulacorthum solani* rare except at Askham Bryan and Sutton Bonington.

P = *Myzus persicae*. *R* = *Aphis rhamni*. *S* = *Macrosiphum solanifolii*.

Aphis traps, 1·3 sq.ft. in area (Broadbent, Doncaster, Hull & Watson, 1948), were operated at all centres except Goole and Reading. The numbers of *M. persicae* caught are shown in Figs. 2 and 3. The numbers of other potato aphides caught were negligible except *Macrosiphum solanifolii* at Askham Bryan (fifty in the season) and Sutton Bonington (twenty-two).

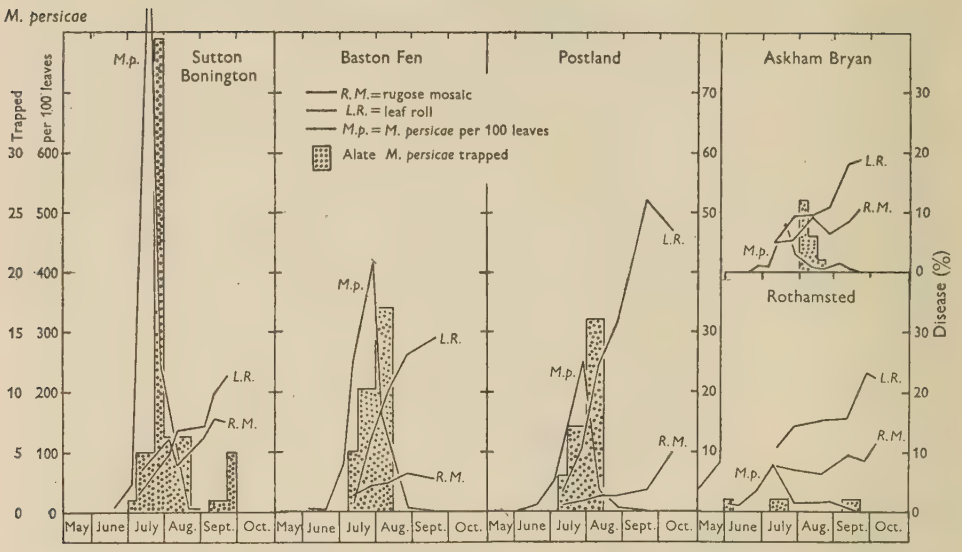

Fig. 2

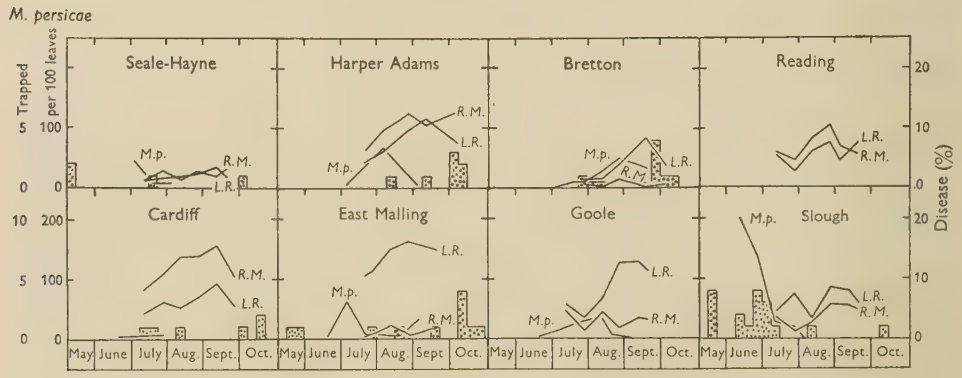

Fig. 3

Figs. 2 and 3. Diagrams showing numbers of *Myzus persicae* on foliage and on traps, and increase of rugose mosaic and leaf roll, from initial zero, at various dates at each centre, 1946.

DATE OF PLANTING

When the mean percentages of disease for each centre were tabulated according to date of planting the averages were as follows:

	1946	
	Before 21 April	After 21 April
No. of centres	8	4
Rugose mosaic	7·2	2·0
Leaf roll	13·7	6·4

The average over the five years 1942–6 for fifty-five fields was very similar, being:

	1942–6	
	Before 21 April	After 21 April
No. of centres	33	22
Rugose mosaic	9·1	3·1
Leaf roll	13·0	6·6

At some centres, such as Sutton Bonington, planting takes place early and there is usually a large spread of disease, while at other centres, where planting is later, there is little spread. Indeed the results over 5 years at many centres, when averaged, show a marked tendency for both diseases to spread less in late-planted crops. This agrees with the results of an experiment on the effects of planting date (Doncaster & Gregory, 1948). A general relation of this type, however, is probably to be expected, as potatoes in northern and upland seed-growing areas would naturally be planted later than in southern ware-growing districts.

Discussion

The experiments in 1946 again showed that nearly half the total amount of virus transmission had taken place soon after the period of tuber-set, and that early lifting of potatoes intended for seed would have benefited only a few crops. At most centres there was more transmission of leaf roll than of virus *Y*. While the amount of early transmission of *Y* and leaf-roll viruses tended to be similar at any one centre, as in previous years the final amount of transmission of these viruses was sometimes widely different.

Figs. 2 and 3 show that there is no close correlation between the spread of either virus disease and maximum number of *Myzus persicae*, either of alatae on the trap or of apterae and alatae on the plants. Nor does converting the count per 100 leaves to the number per plant make the correlation closer, although the number of aphides per plant might be expected to bear more relation to the spread of virus disease by aphides than does the number per 100 leaves. This point will be discussed at length elsewhere.

Table 5. Myzus persicae *and spread of rugose mosaic and leaf roll at Rothamsted and Sutton Bonington* 1945 *and* 1946

	Percentage infection in samples		*M. persicae*	
Centre	Rugose mosaic	Leaf roll	Max no. per 100 leaves	Total trapped during season
1945: Rothamsted	5·4	18·4	111	34
Sutton Bonington	1·2	14·2	8000	892
1946: Rothamsted	8·5	17·2	77	5
Sutton Bonington	11·6	13·7	952	78

The lack of correlation between spread of the two viruses and numbers of *M. persicae* is illustrated by a comparison of data from the years 1945 and 1946 from Rothamsted and Sutton Bonington (Table 5).

In general, those centres with high trap catches in July and August (Askham Bryan, Baston, Postland, Sutton Bonington) showed a pronounced increase in leaf roll in the second half of the season. From this it might appear that leaf roll was spread mainly by alatae, the initial spread being due to the spring migrants, and later spread to alatae produced in or near the crop. Davies (1934), Joyce (1938), Moericke (1941), Doncaster & Gregory (1948) and others have stressed the importance of the early summer migrants in spreading virus disease. Table 6 gives the approximate percentage of leaf roll and rugose mosaic spread at each centre in 1946 before the dispersal of the summer migrants bred on the crop. It has been assumed that a period of about 2 weeks would elapse after infection before the virus would have reached the tubers.

TABLE 6. *Percentage of season's virus spread which occurred before the summer dispersal of* Myzus persicae *alatae from potatoes in* 1946

Centre	Rugose mosaic	Leaf roll
Askham Bryan	63	45
Baston	52	22
Bretton	64	46
Cardiff	80	74
East Malling	32	70
Goole	64	70
Harper Adams	98	85
Postland	35	44
Rothamsted	63	50
Seale-Hayne	61	59
Slough	28	62
Sutton Bonington	53	37
Mean	58	55

Over 50% of the season's spread of both viruses had taken place on the average before the peak of the infestation and the dispersal of summer migrants from the crops. The absence of any considerable increase of rugose mosaic after the initial spread, even at centres with high aphis populations, is further evidence to suggest that alatae are the principal vectors, for most of the plants with rugose mosaic are hidden by healthy plants or are dying by the time alatae are produced within the crop. If spread were largely due to apterae, the fact that the plants were hidden should make little difference and a greater subsequent increase in rugose mosaic disease might be expected. In 1947 plants showing rugose mosaic were marked at Sutton Bonington on 14 July and were dug on 12 August. By the latter date 53% were dead and 47% were more than half dead. This early death of plants with virus *Y* is apparently a result of shading, for plants in the open are dwarfed, but do not die (Doncaster & Gregory, 1948).

M. persicae was numerous at most of the centres in all years. This aphis is known to transmit both viruses, and while the possibility of virus transmission by other aphides cannot be excluded, *M. persicae* is thought to be mainly responsible. The problem that emerges from this series of experiments in which the condition, numbers and positions of both the infectors and the healthy plants have been standardized, is how *M. persicae* can transmit sometimes virus *Y* and sometimes leaf-roll virus. (The numbers of *Aphis rhamni* on the leaves and on the traps were very small in the years 1943–6.) The large differences in the proportions of rugose mosaic and leaf roll found at different centres and in different years is illustrated in Table 7 which gives for 720 plants sampled at each centre the number of new

TABLE 7. *Number of new infections per* 1 *infector* (*adjusted to plot of* 720 *plants*)

	1943		1944		1945		1946	
	Rugose mosaic	Leaf roll	Rugose mosaic	Leaf roll	Rugose mosaic	Leaf roll	Rugose mosaic	Leaf roll
Aberystwyth	0·2	0·9	0·7	1·7	0·06	1·9	—	—
Askham Bryan	—	—	0·8	1·8	0·8	4·0	2·2	3·5
Bangor (Aber)	0·3	0·3	0·2	1·0	—	—	—	—
Baston Fen	—	—	—	—	—	—	1·4	5·5
Bretton	—	—	—	—	—	—	0·2	1·2
Cambridge	7·0	10·1	—	—	—	—	—	—
Cardiff	1·3	3·3	3·0	0·5	0·4	0·6	3·6	1·9
Cockle Park	0·4	0·04	—	—	—	—	—	—
East Malling	2·7	2·3	9·5	2·6	4·1	1·7	0·3	4·1
Goole	—	—	—	—	—	—	0·9	2·6
Harper Adams	5·2	6·6	2·3	2·6	0·9	3·0	3·1	2·2
Kirton	1·3	2·6	—	—	—	—	—	—
Long Ashton	2·4	1·7	4·2	2·4	2·2	1·4	—	—
Okehampton	0·2	0·4	—	—	—	—	—	—
Postland	—	—	—	—	—	—	1·1	8·4
Reading	9·6	8·9	—	—	—	—	2·0	1·6
Rothamsted	1·6	6·3	2·2	6·9	1·7	5·9	2·5	5·1
Seale-Hayne area	2·4	1·0	3·0	2·5	1·2	2·1	0·7	0·6
Slough	—	—	2·8	2·9	3·8	4·8	1·2	1·8
Sutton Bonington	1·2	5·2	3·3	9·6	0·4	4·3	3·5	4·1
Warburton, Ches.	2·8	2·0	—	—	—	—	—	—

infections observed per single infector. It is realized that some of the infections must have come from outside the plot or from volunteer plants, but the table indicates the upper limits for the true spread of infection. The number of new infections to which a single diseased plant gave rise under the conditions of the experiment varied from 0·06 to 9·6 with rugose mosaic and 0·04 to 10·1 with leaf roll.

Evidently counts of aphides cannot give data for forecasting the amount of spread of virus disease in a field crop. The amount depends on unexplained factors, possibly affecting the movement or efficiency of vectors, or the infectiousness of

infectors or the susceptibility of the receptor plants. The presence of a sufficient number of *Myzus persicae* must be only one of several limiting factors.

In this series of experiments the traps have proved useful in showing the time of the dispersal of summer migrants from the crop. There has also been some relation between the number of *M. persicae* caught on the trap at this time and the size of the population on the field, especially when expressed as number per plant. A close correlation cannot be expected, because aphides from other crops will be caught on the traps as they pass over the field. The traps have not indicated the size of the spring migration to the potatoes, as at this time *M. persicae* is present only in small numbers. Trapping is worth while where the crop cannot be visited at regular intervals, but gives little or no additional information when the crop can be inspected frequently.

Data now exist for a review of the infection gradients over the period 1943–6. The mean percentage of rugose mosaic and leaf roll in the nearest, intermediate and farthest positions from the infector strip for the first two and last two sampling occasions in each of the years 1943–6 is shown in Fig. 4. (Occasions 3 and 4 have

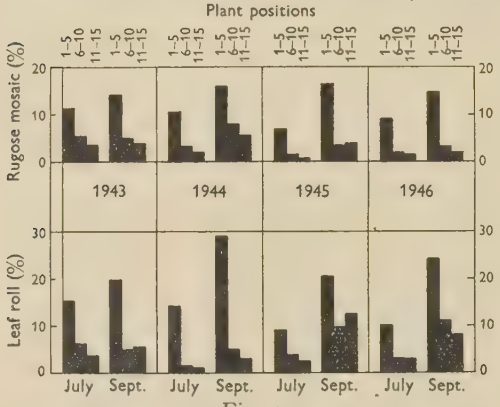

Fig. 4

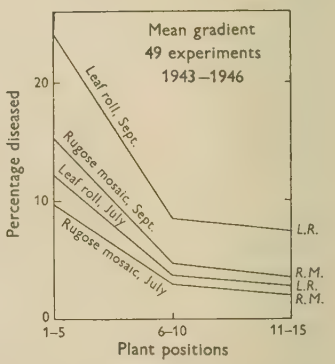

Fig. 5

Fig. 4. Histograms showing infection gradients (percentage rugose mosaic and leaf roll) at various distances from the source of disease in July and September 1943 to 1946.

Fig. 5. Mean infection gradients of rugose mosaic and leaf roll based on forty-nine experiments (1943–6) in July and September.

been omitted because the asymmetrical sampling procedure of the earlier years may have led to abnormally high mid-season values, though there is little internal evidence that this happened.)

There is a general similarity between the gradients of rugose mosaic and leaf roll. When mean values for all years are considered, the gradients of both diseases are somewhat flatter at the beginning than at the end of the season (Fig. 5). This suggests that the vectors responsible for late infections tend to move shorter distances than

those active earlier in the season. This might be due either to late spread being largely the work of apterae, or to the mean flight length of alatae being different in spring and summer. If the frequency distribution of range-distances of vectors had remained uniform throughout the season, it would be expected that towards the end of the season gradients expressed as percentage diseased plants would get flatter, because of the increasing likelihood of plants already infected receiving a second inoculation with the same virus, which would not show in the records. Records of percentage presence or absence on a plot or plant give a false gradient, depending on intensity of infection and size of plot, and to get a true measure of the gradient of incidence of a virus or organism it is necessary first to apply Thompson's transformation (Gregory 1945, 1948). When this is done to the data in Fig. 5 the end of season gradient for leaf roll becomes still steeper. This increase of gradient with time has apparently not been reported before.

We wish to thank the following workers who carried out the experiments at the various centres: Dr I. F. Storey and Mr G. D. Glynne Jones (Askham Bryan and Goole); Mr J. Rees and Dr E. E. Edwards (Cardiff); Dr R. V. Harris and Dr I. W. Prentice (East Malling); Mr N. C. Preston and Mr H. F. Newton (Harper Adams); Mr W. Buddin and Dr A. G. Walker (Reading); Mr A. Beaumont and Mr H. G. Morgan (Seale-Hayne); Prof. W. Brown and Miss B. N. Hawkey (Slough); Mrs N. McDermott and Mr A. Roebuck (Sutton Bonington). The work was carried out on behalf of the Agricultural Research Council.

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THE EFFECT OF ROGUING ON THE SPREAD OF VIRUS DISEASES IN POTATOES AT ROTHAMSTED IN 1946

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(With 2 Text-figures)

Experiments on roguing virus-diseased plants from plots of Majestic potatoes, which have been in progress since 1943, were continued in 1946. Plots were rogued in mid-June, early and late July, and plants were lifted from these plots at the end of July, August and September respectively. Roguing had little effect in reducing the spread of rugose mosaic (caused by potato virus *Y*). The spread of leaf roll was reduced to half that on unrogued plots by roguing on 14 June. Later roguing did not reduce the spread of leaf roll, unless combined with early lifting. Early lifting increased the effect of early roguing. In spite of these results roguing main crop potatoes in the south of England is not considered a practical control measure.

Results of experiments on the effect of roguing rugose mosaic (caused by potato virus *Y*) and leaf-roll plants from Majestic potatoes at Rothamsted in 1943, 1944 and 1945 have already been reported (Doncaster & Gregory, 1948). The removal of diseased plants coming from infected tubers in July had no effect on the health of tubers saved from the crop. There was, however, an indication in 1944 that mid-June roguing had been early enough to prevent a large proportion of the spread of virus *Y*, although it had no effect on leaf roll. An experiment in 1946 was designed to test the effect of several roguing dates on the health of tubers dug from a Majestic crop at the end of the months of July, August and September respectively.

Design of experiment

The experiment was in a field of potatoes (western half of Bones Close) with the following history: 1942, old woodland reclaimed; 1943, wheat; 1944, wheat; 1945, kale and barley.

The 1946 experiment comprised sixteen plots, each containing 430 plants of Scotch Stock Seed Majestic potatoes and twenty-five infector plants, surrounded by non-experimental potatoes of Scotch A certificate standard, and by other potato experiments to the east. Each plot was 13 rows × 35 plants. Rows 3, 5, 7, 9 and 11 contained infector plants as in Fig. 2. An adequate number of tubers infected singly with virus *Y* and leaf roll was not available so tubers from a stock of King Edward grown three times in England were used as sources of virus infection. Of

the plants in this stock, 69% were infected with both viruses, 14% with rugose mosaic only and 13% with leaf roll only.*

The main plots, which were arranged in a 4×4 Latin square (Fig. 1), were rogued on various dates. Single rows within main plots were sampled on various dates. At the time of roguing the haulm of the diseased King Edward was pulled, the parent sett and tubers dug out and the whole placed at once in a sack and taken off the field. When sampling, a single tuber was dug from each of the twenty originally healthy Majestic plants in the row selected. The ten tubers from plants next to the five infectors, position 1, were placed in one bag, and those from position 2 in another. The sample tubers were stored and subsequently planted at the Nottingham University College School of Agriculture, Sutton Bonington, and scored for virus diseases in June and July 1947.

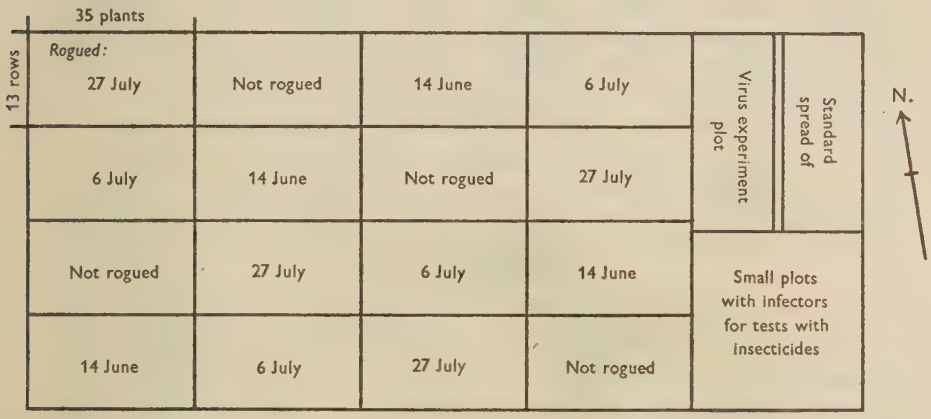

Fig. 1. Plan of 4×4 Latin square roguing experiment, and adjacent experiments, Rothamsted 1946.

In the 1946 experiments 'infector plants' were in the ratio of one to every four healthy plants in the sampling area, and all plants sampled were either next to (position 1) or one plant away from an infector (position 2). The proportion of infectors was thus higher than in 1943–5, when there was one infector to ten healthy plants.

Results of experiment

For comparison with earlier years sampling of the five plants on each side of an infector was also done in the guard areas between plots at the beginning of October 1946 ('infector unit' method). The infectors at each end of the rows provided half a unit each (Fig. 2). Table 1 shows the percentage infection in different positions in the infector units for the years 1943–6. The rugose-mosaic gradient was irregular

* This is precisely the percentage of mixed infections that would have been expected if infection of a plant with one virus did not affect its chances of becoming infected with the second.

in 1946 and resembled results obtained in Lincolnshire potato fields in 1941. The leaf-roll gradient for 1946 was almost identical with that obtained at Rothamsted in 1945 on the eastern half of the same field.

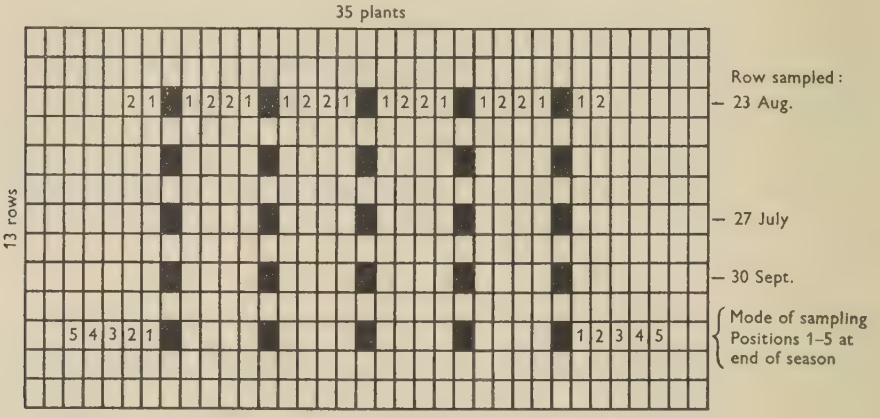

Fig. 2. Plan of a single plot, showing position of King Edward infector plants, and mode of sampling adjacent healthy plants.

TABLE 1. *Rugose-mosaic and leaf-roll infections in plants* 1–5 *from the infectors at Rothamsted*, 1943–6

(Infector unit sampling)

Disease	Year	Approx. no. of plants sampled	Percentage infected plant positions 1	2	3	4	5
Rugose mosaic	1943	800	46	16	6	4	3
	1944	960	54	23	19	9	5
	1945	640	38	7	5	3	0·8
	1946	760	21	10	3	6	7
Leaf roll	1943	800	51	31	19	13	11
	1944	960	82	59	47	33	18
	1945	640	45	24	14	11	8
	1946	760	43	22	19	10	9

The following records on the aphis infestation were obtained in connexion with another experiment on the same field which has been described by Broadbent & Gregory (1948). The first *Myzus persicae* was caught on the trap during the first week in June and at the same time the first individuals were found on the potato foliage. A maximum of 77 per 100 leaves was reached early in July, and a small infestation persisted throughout August. Only four more *M. persicae* were trapped during the rest of the season. Very small infestations of *Aphis rhamni* and *Macrosiphum solanifolii* persisted from July to September.

The numbers of rugose-mosaic and leaf-roll infections resulting from each treatment are shown in Table 2, together with the number out of a possible forty

surviving plants on which the reading was based (the four replicates have been pooled). Tables 4 and 5 show the percentage rugose mosaic and leaf roll according to treatments.

TABLE 2. *Effect of roguing and date of lifting on spread of rugose mosaic and leaf roll in Majestic potatoes at Rothamsted* 1946 (*four replicates pooled*)

	Lifting dates						
	27 July		23 Aug.		30 Sept.		
Positions ...	1	2	1	2	1	2	Totals
Roguing date:							
14 June	3 : 5* / 40	1 : 2 / 38	8 : 6 / 37	5 : 11 / 38	5 : 7 / 40	5 : 11 / 38	27 : 42 / 231
6 July	2 : 16 / 37	3 : 9 / 38	7 : 17 / 38	1 : 9 / 38	4 : 23 / 40	1 : 14 / 39	18 : 88 / 230
27 July	5 : 15 / 40	8 : 11 / 39	8 : 16 / 38	4 : 8 / 39	8 : 23 / 38	3 : 16 / 40	36 : 89 / 234
Not rogued	4 : 17 / 38	4 : 9 / 40	4 : 22 / 38	1 : 13 / 34	6 : 24 / 40	5 : 9 / 38	24 : 94 / 228
Totals	30 : 84 / 310		38 : 102 / 300		37 : 127 / 313		105 : 313 / 923

* Rugose mosaic : Leaf roll / Total

The percentage of each disease in positions 1 and 2 for various roguing and lifting dates is shown in Table 3. As would be expected, the gradient here is flatter between positions 1 and 2 than in the 'infector unit' samples in Table 1. This is probably because plants in position 2 in the roguing experiments were usually simultaneously in position 3 relative to another infector, and so would have an increased chance of infection.

TABLE 3. *Percentage rugose mosaic and leaf roll in positions 1 and 2 (replicates pooled)*

	Rugose mosaic (%)		Leaf roll (%)	
Positions ...	1	2	1	2
Roguing date:				
14 June	13·7	9·8	15·2	21·0
6 July	11·3	4·4	48·6	27·8
27 July	18·1	12·7	46·6	29·7
Not rogued	12·1	8·9	54·3	27·6
Lifting date:				
27 July	9·0	10·3	34·2	20·0
23 Aug.	17·9	7·4	40·4	27·5
30 Sept.	14·5	9·0	48·6	32·2
Mean	13·8	8·9	41·2	26·6

The amount of virus *Y* transmitted was small in comparison with leaf roll (Table 4) and its variability from plot to plot was high. No convincing effect of roguing is shown for virus *Y*.

TABLE 4. *Percentage rugose mosaic (positions 1 and 2 and replicates pooled)*

	Lifting dates			
	27 July	23 Aug.	30 Sept.	Mean
Roguing dates:				
14 June	5·1	17·4	12·8	11·7
6 July	6·7	10·5	6·3	7·8
27 July	16·7	15·6	13·9	15·4
Not rogued	10·2	6·9	14·1	10·5
	9·7	12·7	11·8	11·4

TABLE 5. *Percentage leaf roll (positions 1 and 2 and replicates pooled)*

	Lifting dates			
	27 July	23 Aug.	30 Sept.	Mean
Roguing dates:				
14 June	9·0	22·7	23·1	18·2
6 July	33·3	34·2	46·9	38·3
27 July	32·9	31·2	49·4	38·0
Not rogued	33·4	48·6	42·4	41·2
	27·1	34·0	40·6	34·0

With leaf roll, clear trends are obvious from Table 5. Plots rogued 14 June, and lifted 27 July, showed about one-quarter of the season's maximum infection. Plots rogued 14 June but lifted later showed about one-half maximum infection. Roguing 6 July or 27 July gave three-quarters of the season's maximum infection with leaf roll in the July or August lifting occasions, and were both up to maximum infection by the end of September, presumably by spread from plants infected during the current season. The 'not rogued' plots showed the full season's maximum infection by the time of the August lifting. (For the 27 July lifting date, plots rogued 27 July and 'not rogued' were in effect identical in treatment, and the score for leaf roll was similar.) Information on the behaviour of leaf roll in the field is still insufficient to permit any interpretation of these interactions.

The experiment indicates how a combination of early roguing and early lifting would substantially reduce the amount of leaf roll in a crop grown specially for seed. The 1946 experiment does not conflict with the conclusion already reached (Doncaster & Gregory, 1948) that roguing diseased plants grown from infected tubers is of little value in improving seed tubers from crops grown for ware in the south of England, both because loss of yield resulting from lifting in July is substantial, and because symptoms are seldom well enough developed to allow roguing in mid-June. Reasons have already been given why the conclusion drawn from these experiments carried out in ware-growing districts should not apply to seed-growing areas in

which roguing has long been practised and has led to a valuable improvement of potato stocks.

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THE MULTIPLE-INFECTION TRANSFORMATION

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(With 1 Text-figure)

Attention is drawn to the possibility of misinterpreting results which may arise when the incidence of an organism, or any other event, is expressed as percentage presence or absence on plots of standard area. Disease gradients when expressed as percentage presence are shown to be artificially flattened. The amount of flattening depends on incidence of the disease and the size of plot chosen. A table is given by which the necessary transformation can be made when the organism or event is distributed at random. Examples are given of leaf diseases: (1) distributed at random (*Phytophthora infestans*); and (2) aggregated (*Gymnosporangium juniperi-virginianae* and *G. clavariaeforme*). Methods of detecting and measuring aggregation are briefly discussed.

Records of the incidence of an organism, a disease or parasite on a crop, or any other event, are commonly expressed in two distinct ways: (1) the number of organisms, lesions, etc. per unit area; and (2) the percentage of unit areas 'infected', irrespective of the number of organisms which have brought about the infection. For plant diseases the unit chosen may be a measured area, but it is more often an organ such as a leaf, or even a whole plant. For convenience the unit will be called a plot. The incidence of a systemic disease is nearly always expressed as a percentage of the total plants. As pointed out by Ashby (1936), the relation between these two methods of expressing incidence of infection is not linear. Aberrant results may be obtained if percentage data are used in the study of plant-disease gradients to infer variations of spore density or numbers of aphis punctures. It is first necessary to apply to the data what I have elsewhere termed 'Thompson's transformation' (Gregory, 1945), from the method first worked out for insect parasites by Thompson (1924). The method was developed for ecological studies on *Ribes* by Fracker & Brischle (1944), who tabulated the transformation. Table 1 below incorporates their data, by permission, and adds new values between 80 and 99·9%.

The need for the transformation may be illustrated by considering a hypothetical case of 100 potato plants, uniformly susceptible and exposed to infection by *Phytophthora infestans* from a distant source. The first spore that causes an infection must obviously infect 1% of the plants. A second infection, so long as it falls *at random* among the 100 plants, will have one chance in 100 of alighting on the plant already infected, instead of infecting a second plant. As the percentage of infected plants rises, the probability that each additional infection falls on a plant already infected, thus producing no increases in percentage plants infected, increases greatly. When 99% of the plants are infected, another infection will have only one chance

in 100 of falling on the single remaining healthy plant. Different parts of the percentage range, therefore, correspond to very different spore densities per unit area, and the transformation can be neglected only in the lower percentage categories.

TABLE 1. *Multiple infection transformation: percentages to infections (calculated from 5-figure log tables)*

y%	x	y%	x	y%	x	y%	x	y%	x	y%	x
1	1·00	23	26·14	44	57·98	65	105·0	83	177·2	93·5	273·3
2	2·02	24	27·44	45	59·78	66	107·9	83·5	180·2	94	281·3
3	3·05	25	28·77	46	61·62	67	110·9	84	183·3	94·5	290·0
4	4·08	26	30·11	47	63·49	68	113·9	84·5	186·4	95	299·6
5	5·13	27	31·47	48	65·39	69	117·1	85	189·7	95·5	310·1
6	6·19	28	32·85	49	67·33	70	120·4	85·5	193·1	96	321·9
7	7·26	29	34·25	50	69·31	71	123·8	86	196·6	96·5	335·2
8	8·34	30	35·67	51	71·33	72	127·3	86·5	200·2	97	350·7
9	9·43	31	37·11	52	73·40	73	130·9	87	204·0	97·5	368·9
10	10·54	32	38·57	53	75·50	74	134·7	87·5	207·9	98	391·2
11	11·65	33	40·05	54	77·65	75	138·6	88	212·0	98·5	420·0
12	12·78	34	41·55	55	79·85	76	142·7	88·5	216·3	99	460·5
13	13·93	35	43·08	56	82·10	77	147·0	89	220·7	99·1	471·0
14	15·08	36	44·63	57	84·40	78	151·4	89·5	225·4	99·2	482·8
15	16·25	37	46·20	58	86·75	79	156·1	90	230·3	99·3	496·2
16	17·44	38	47·80	59	89·16	80	160·9	90·5	235·4	99·4	511·6
17	18·63	39	49·43	60	91·63	80·5	163·5	91	240·8	99·5	529·8
18	19·85	40	51·08	61	94·16	81	166·1	91·5	246·5	99·6	552·1
19	21·07	41	52·76	62	96·76	81·5	168·7	92	252·6	99·7	580·9
20	22·31	42	54·47	63	99·43	82	171·5	92·5	259·0	99·8	621·5
21	23·57	43	56·21	64	102·2	82·5	174·3	93	265·9	99·9	690·8
22	24·85										

With acknowledgements to Drs S. B. Fracker and H. A. Brischle of the United States Department of Agriculture.

Thompson applied the Poisson distribution to the problem of multiple infection and showed that if N = number of hosts available, and y = average number of hosts infected after the deposition at random among the hosts of x parasites, then

$$y = N(1 - e^{-x/N}),$$

or

$$x = N \log_e 10 \, [\log_{10} N - \log_{10} (N - y)].$$

When y is a percentage, then $N = 100$, and x may readily be calculated from $x = 230{\cdot}258 \, [2 - \log_{10} (100 - y)]$. In Table 1, x has been calculated for values of y varying from 1 to 99·9%. It will be seen that, while only one infection is required to bring about an increase from 1 to 2%, the increase from 98 to 99% requires sixty-nine infections (that is 460 − 391).

THE INTERPRETATION OF PLANT-DISEASE GRADIENTS

The reason for making this transformation arises from the fact that one would expect to find the rules relating the dispersion of an organism with distance simpler if actual numbers were used in place of percentages. The use of observed percentages of individuals infected may give a distorted picture of the dispersion of the

fungus, virus or insect unless the multiple infection transformation is applied. For example, Posey & Ford (1924) give the percentage of white pine trees (*Pinus strobus*) infected with blister rust (*Cronatium ribicola* Fischer) in 100 ft. zones from the source of infection which consisted of a group of fifteen bushes of black currant (*Ribes nigrum*). The gradient of percentage infection at increasing distances from the source is much flatter than the corresponding gradient of the relative densities of spores at these distances when calculated from the multiple infection transformation (Table 2). The amount of flattening will obviously depend on the level of disease incidence and on the size of the organ or plot chosen.

TABLE 2. *Posey & Ford's* (1924) *data on decrease of blister rust with increasing distance from source*

	Observed percentage trees infected		Expected number of infections per 100 trees	
Distance (ft.)	East	West	East	West
0–100	65	88	105	212
100–200	15	58	16	87
200–300	4	45	4	60
300–400	2	26	2	30
400–500	3	25	3	29
500–600	3	19	3	21
600–700	2	10	2	10
700–800	1	6	1	6

Wilson & Baker (1946) have shown that this and many other plant-disease gradients expressed as percentages can be fitted by an empirical equation with a single parameter a. In different gradients a was found to vary from 6·5 to 0·21, a range of over thirty times. Similar empirical equations with highly variable parameters have been developed by Wolfenbarger (1946). The range could probably be narrowed considerably if the data were corrected for multiple infections. This may be done legitimately if the infections are distributed at random among the hosts.

RANDOM AND NON-RANDOM DISTRIBUTION

If the distribution of infections is at random among the hosts a straight line will be obtained when the logarithm of the percentage uninfected (100 − % infected) is plotted against the number of infections (x). The slope of the line is given by $b = -0{\cdot}004343$. Blackman (1942), in ecological studies of flowering-plant communities, found that the occurrence of plants on quadrats may depart from the expected random distribution and yet still give a reasonably straight line when plotted as before. The slope of the observed line, however, differs from that of the expected line. Blackman's 'correction factor', the ratio of the slope of the expected line to the slope of the observed line $\left(K = \frac{b_{\text{exp.}}}{b_{\text{obs.}}}\right)$, then gives a useful measure of the departure from a random arrangement. Various other mathematically more plausible

formulations of this deviation have been attempted and are reviewed by Fracker & Brischle (1944). Non-random distribution may be due to a number of factors, such as repulsion between individuals ('under-dispersion' as understood by ecologists), and K will then be less than unity. Aggregation ('over-dispersion'), on the other hand, leads to K greater than unity. Aggregation may be due to such causes as local spread of infection, progeny remaining near the parent, or to local differences in susceptibility. van der Plank (1946) has given a useful test for detecting aggregation in the field.

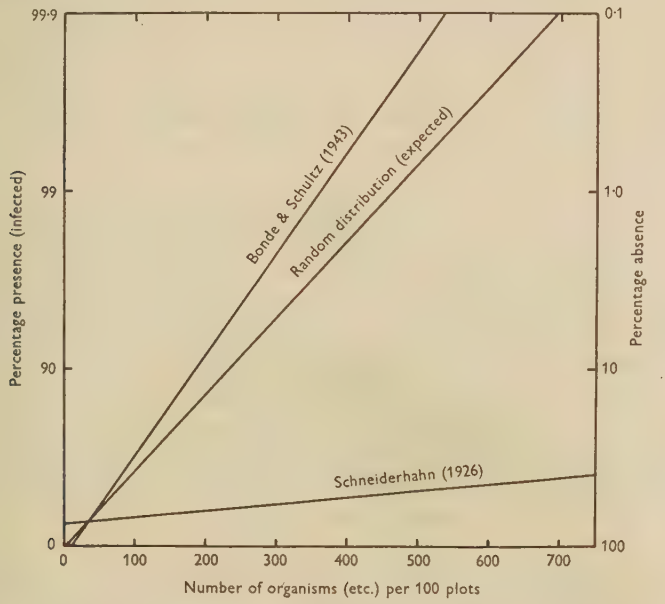

Fig. 1. Relation between number of organisms (or other event) per 100 plots, and percentage plots on which the organism is present (or absent). Expected random distribution, and observed distributions of *Phytophthora infestans* (Bonde & Schultz, 1943) and *Gymnosporangium juniperi-virginianae* (Schneiderhahn, 1926).

Under appropriate conditions, the observed scatter of lesions may be very close to that expected from a random distribution. Bonde & Schultz (1943) give counts both of the percentage of potato plants infected and the numbers of lesions per 100 plants in positions at several distances from potato refuse piles which were acting as sources of spores of *Phytophthora infestans*. The linear regression calculated from their data and plotted in Fig. 1 has slope $b = -0{\cdot}00587$. This is close to the expected random distribution which has slope $b = 0{\cdot}00434$. $K = \frac{0{\cdot}00434}{0{\cdot}00587} = 0{\cdot}74$, and shows slight repulsion. The observed line is not, however, significantly different

from the expected line ($t = 0{\cdot}352$, not significant at $P = 0{\cdot}05$, for $n = 3$). Thus, although when the various distances are compared the lesions show a gradient in relation to the source, their scatter at any one distance is apparently random.

In some cases the distribution differs widely from the random arrangement. Schneiderhahn (1926) gives percentages of apple leaves infected with *Gymnosporangium juniperi-virginianae*, and also counts of the average number of rust spots per leaf. The apples were at various distances from groups of juniper trees in different localities, which were acting as sources of sporidia. The linear regression, calculated from the data plotted in Fig. 1, deviates widely from the expected random arrangement $b = -0{\cdot}000329$ ($K = 13{\cdot}2$; $t = 714$, significant at $P = 0{\cdot}001$, $n = 8$). There is thus very strong evidence of aggregation of lesions on the infected leaves at the higher levels of infection. At lower levels of infection agreement is better, and the data ought to be fitted with a curved line passing through the origin.

As local multiplication on apple is precluded by the life cycle of the fungus the deviation is probably due to individual leaves of a group having wide differences in susceptibility. Schneiderhahn, in fact, observed that as the heaviest spore production from telia on the junipers occurred before most of the apple leaves had developed, a large proportion of the leaves would therefore escape exposure to infection.

I observed a similar effect with *G. clavariaeforme* at Gustardwood Common, Hertfordshire, in the spring of 1947. The area investigated was a heath containing numerous clumps and isolated bushes of *Juniperus communis* L. and *Crataegus monogyna* Jacquin. At the end of April and beginning of May most of the junipers had galls producing telial horns. On 25 May *Crataegus* shoots were removed from bushes at various distances from junipers, and the incidence of lesions with pycnidia recorded on approximately 2500 leaves (Table 3). The ground was uneven and the distribution of the source bushes irregular.

TABLE 3. *Relation between percentage leaves infected with* Gymnosporangium clavariaeforme *and number of lesions per* 100 *leaves*

Approximate distance (m.) from nearest juniper	Percentage leaves infected	Lesions per 100 leaves	
		Observed	Expected if distributed at random
1	33·7	94·3	41
5	7·8	10·1	8
6 and 7	9·2	10·5	—
20	2·0	5·0	—
70	10·1	11·7	—

(The high incidence at 70 m. was evidently due to topographical irregularities.) At approximately 1 m. the number of infections observed was over twice the number expected from the proportion of leaves infected. This set of data, together with Schneiderhahn's, indicates that in *Gymnosporangium* the distribution of lesions on

the Rosaceous host is not at random. Whatever the explanation, the data are sufficient to demonstrate the difficulty of inferring gradients of spore density when only the percentage of hosts infected (or uninfected) is known. It is necessary not only to apply the multiple infection transformation, but also to know the correction factor, such as Blackman's K, in cases where the scatter is not at random. If a high value of K were to apply to Posey & Ford's distribution (Table 2) a very different gradient of spore density would result. At present information on these distributions is scanty. The collection of more field data in which numbers of infections as well as percentages are recorded is a prerequisite to the understanding of factors controlling the dispersion of infection.

Criticism and advice from Mr F. J. Anscombe of the Statistical Department during the preparation of this paper are gratefully acknowledged.

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PHOTOSYNTHESIS AND PREDISPOSITION OF PLANTS TO INFECTION WITH CERTAIN VIRUSES

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The effects on susceptibility to infection with certain viruses of subjecting plants to various periods of darkness or reduced illumination before and after inoculation were tested. The viruses and hosts used were a tobacco necrosis virus in French bean and tobacco; tomato aucuba mosaic virus in tobacco; and tobacco mosaic and tomato bushy stunt viruses in *Nicotiana glutinosa*. All the virus-host combinations give necrotic local lesions, and susceptibility was measured by local lesion counts. Susceptibility was consistently increased by pre-inoculation treatments of host plants, whereas post-inoculation treatments had relatively little effect, but most often decreased susceptibility.

Short periods in the dark produced similar responses to longer periods in shade, but the different plants varied in their response to, and tolerance of, darkness. The maximum number of lesions was usually obtained with bean plants kept for only 24 hr. in the dark before inoculation, but with tobacco plants susceptibility increased with increasing time in the dark up to 5 days.

It is suggested that the successful establishment of infection occurs in two stages, the first of which is affected by the accumulation of photosynthetic products. Whether these products confer resistance by increasing cell turgor or by reacting specifically with virus particles is unknown, but sap from plants in the light possesses no greater virus-inhibiting power than sap from plants kept in the dark.

We have shown previously (Bawden & Roberts, 1947) that during the summer months the susceptibility of plants to infection with certain viruses is increased if they are kept under conditions of reduced light intensity. In those experiments test plants were maintained under shade from the seedling stage onwards and were kept in the same conditions after they were inoculated as before. Hence there were no results to indicate the relative importance of reducing the light intensity before and after inoculation; nor did the results show how susceptibility was affected by different periods of treatment or by excluding different amounts of light. The experiments described in the present paper were made to provide some of this information. The results show that the conditions to which plants are exposed before they are inoculated are much more important than the treatment immediately after inoculation, and that relatively short periods in the dark considerably increase their susceptibility.

Materials and methods

Susceptibility was measured by counts of local lesions and the virus-host combinations used were those that gave necrotic local lesions only, with no systemic infection. The viruses and their hosts were the Rothamsted culture of tobacco necrosis virus on French bean, *Phaseolus vulgaris*, var. Prince, and tobacco, *Nicotiana tabacum*,

var. White Burley; tomato bushy stunt and tobacco mosaic viruses on *N. glutinosa*; and tomato aucuba mosaic virus, a strain of tobacco mosaic virus, on tobacco. Purified virus preparations were used as inocula, and the inoculations were made by rubbing the upper surfaces of the leaves as uniformly as possible with the forefinger wet with the inoculum. Two dilutions of inoculum, differing by a factor of ten, were used in each experiment, the more dilute being applied to the left-hand halves of the leaves and the more concentrated to the right-hand halves. This was done to obtain results in the range in which the numbers of lesions produced would be approximately proportional to the susceptibility of the leaves. From the nature of the experiments, different treatments had to be applied to whole plants, a fact that introduces serious errors because of differences between the susceptibility of individual plants. To reduce such variations as far as possible, at least eight bean plants were used for each treatment in every experiment, and at least five tobacco or *N. glutinosa* plants. All the plants to be used in any one experiment came from the same batch of seedlings, were raised as far as possible under the same conditions and were at all times kept in the same glasshouse. Any plants that obviously differed from the majority were rejected, and from the seemingly uniform remainder the plants to be used for any particular treatment were selected at random. Bean plants were used about a fortnight after sowing, when the first-formed leaves were well grown and the first trifoliate leaves were beginning to develop; only the first-formed leaves were inoculated. Tobacco and *N. glutinosa* plants were used when they had produced from eight to ten leaves; three similarly positioned, well-developed leaves on each tobacco plant were inoculated, and the six middle leaves were inoculated on the *N. glutinosa* plants. With all the host plants, the leaves not to be inoculated were removed immediately before the time of inoculation.

The experiments were all made between April and September 1947, a period during which there were unusually prolonged spells of warm sunny weather. The differential light conditions were provided by enclosing the treated plants in light wooden frames 24 in. high and with sides 34 in. long, which were covered with hoods of single or double thickness of closely woven white muslin or with opaque black cloth. The single and double hoods of muslin reduced the light intensity to approximately one-third and one-ninth of that incident on plants outside, and the conditions in these cages will be called shade and double shade respectively. The third type of cover excluded all direct light, and the conditions in these cages will be called darkness, although a small amount of light did come through the spaces between the slats of the greenhouse bench on which the cages and the pots were standing. The air temperature within the cages did not vary from that of the glasshouse by more than 2° C. The conditions that will be referred to as light were the ordinary fluctuating conditions of the glasshouses. The plants were rarely exposed to direct sunlight for any long periods, because the glasshouse blinds were drawn whenever normal practice required it. No illumination was provided at night, so that no plants were continuously in the light, and periods referred to as time in the

light include the normal alternations of day and night. It was found that a few hours in the dark could sometimes increase susceptibility appreciably, so that it seemed likely that the time of day at which inoculations were made might influence the response of plants to treatments and, if varied, might cause variations between the results of successive experiments. To minimize this possibility, all inoculations were made in the middle of the afternoon, between 15.00 and 17.00 G.M.T., when the control plants in the light had received at least 10 hr. of continuous daylight. Plants were not removed from the cages until immediately before they were to be inoculated and they were placed in the selected conditions immediately afterwards.

The effects of treatments of healthy plants

The prolonged periods of shading employed in the earlier tests produced considerable effects on both the appearance and constitution of the plants (Bawden & Roberts, 1947). The leaves of the shaded plants were deeper green and larger though thinner than those of the controls; they were also more succulent and fragile, and felt much softer. The relatively short periods of shading used in the present experiments produced no such obvious effects in the appearance of plants. Presumably they did reduce the dry weight of the leaves, but this was not tested, and there was no increase in fragility or change in texture that was apparent to the touch. Nor did the periods spent in the dark affect the 'feel' of the leaves, although, as would be expected, these had deleterious effects when prolonged. The tolerance of all the host plants used to continuous darkness increased with increasing age, but there was considerable difference between the different species. Bean plants, perhaps because they were the youngest used, were most affected, and sometimes suffered obvious damage from 48 hr. in the dark cages; the young tri-foliate leaves became peppered with necrotic spots, and on the first-formed leaves there sometimes developed brown areas with a scorched appearance. *N. glutinosa* showed no ill effects from 2 days, but 3–4 days usually caused the production of necrotic lesions on the upper leaves. Tobacco plants were the least affected and could tolerate 6 days without becoming necrotic, although during this time the leaves became abnormally erect and lost some of their green colour. Such plants regained their normal appearance after a few days under ordinary glasshouse conditions.

No detailed analyses were made of the effects of periods of darkness on the composition of whole leaves, but sap from some plants was examined. Comparable leaves were picked from tobacco plants kept for 96 hr. in the dark cages and from plants growing in the light; they were minced separately by passage through a meat mincer, and their sap expressed through muslin. The nitrogen contents of the two lots of sap were the same, viz. 1·51 mg./ml., but the solid content of the sap from the plants in the light was 74 mg./ml. and that of the plants from the dark only 34·6 mg./ml., a difference accounted for by the reduction in carbohydrate from 45 mg./ml. in the former to 5 mg./ml. in the latter. Essentially similar differences were obtained when leaves from bean plants kept in the light were compared

with those from plants kept for 48 hr. in the dark, the nitrogen contents of the two lots of sap being 3·5 and 3·3 mg./ml., their solid contents 90 and 60 mg./ml., and their carbohydrate contents 43·5 and 7·3 mg./ml. Carbohydrate was measured colorimetrically by the orcinol-sulphuric acid method with glucose as the standard.

Tobacco necrosis virus

Most experiments with the tobacco necrosis virus were made to test the effect on the susceptibility of bean plants produced by periods in the dark before and after inoculation. Qualitatively the results were extremely uniform, but there were considerable differences between the quantitative responses obtained by the same treatment in different experiments. Much of this variation probably results from differences in the intrinsic susceptibility of the different batches of plants inoculated at different dates, from differences in their ages (both physiological and actual), and from differences in the light intensity to which the plants were subjected before and during the course of the experiment. Other things being equal, it is to be expected that the response to a period in the dark, which is measured by the relative number of lesions produced on the treated and control plants, would be different during a period of bright sunshine and one of dull weather. Also, the weather before the plants were placed in the dark might have a considerable influence on the response to darkness. In spite of the variations, however, in all the experiments a period in the dark before inoculation increased susceptibility, whereas one after inoculation did not and most often led to a decrease in susceptibility.

In different experiments, the average number of lesions per half-leaf was increased by from two to nine times by keeping the plants for 24 or 48 hr. in darkness immediately before inoculation. In seven out of nine experiments, the plants in the dark for 24 hr. produced more lesions than those in the dark for 48 hr., and from the average of all experiments the plants treated for 24 and 48 hr. respectively produced 418 and 290% of the numbers of lesions produced by control plants kept in the light. A few experiments to test the effect of shorter periods of darkness before inoculation showed that 1 hr. produced no appreciable change, but that on the average 2, 4 and 8 hr. increased the numbers of lesions to 150, 200 and 250% of those produced by the control plants. Because of the harmful effects on bean plants of prolonged periods in the dark, only one test was made with a pre-inoculation treatment of more than 48 hr. The results are given in Table 1, and show that, although the plants treated for 72 hr. produced more lesions than the controls, they produced fewer than those treated for either 24 or 48 hr.

Table 1 also shows that keeping the plants in darkness after they had been inoculated produced no increase in susceptibility. The results with the post-inoculation treatments from different experiments have varied more than with pre-inoculation treatments but, on the average, placing plants in the dark has resulted in a fall in the numbers of lesions to approximately 75% of the number produced on the control

plants. In twelve out of fifteen experiments, the control plants have produced more lesions than those placed in the dark after inoculation for either 24 or 48 hr.; in one (Table 1) the 24 hr. treatment gave slightly more lesions, and in the other two approximately twice as many lesions were obtained on the plants placed in the dark as on the controls. Shading after inoculation has given similar erratic results. In most experiments, shade, like darkness, has caused a slight fall in the number of lesions, but in some it has produced increases. This effect has been specially noticeable when the plants treated were those that had also been in the single or double shade cages before they were inoculated. The reasons for the variable results are unknown, but they may result from variations in the glasshouse conditions at the time of different experiments. The conditions to which plants in the cages were exposed after inoculation were relatively constant compared to those in the light, and it is possible that experiments in which caging after inoculation produced an increase in the numbers of lesions were those in which the inoculated plants placed in normal glasshouse conditions were exposed to unusually bright light. Immediately after

Table 1. *The effect of periods of darkness on the susceptibility of bean plants to a tobacco necrosis virus*

Treatment of plants		Average no. of lesions per half-leaf with inoculum at	
Before inoculation	After inoculation	10 mg./l.	1 mg./l.
Light	Light	26	6
72 hr. dark	Light	70	28
48 hr. dark	Light	111	35
24 hr. dark	Light	98	45
Light	24 hr. dark	32	7
Light	48 hr. dark	18	4

inoculation leaves wilt very easily, and exposure to bright light can cause serious wilting or even scorching, especially if the plants have been maintained previously in shade, which could lead to the death of some cells and so prevent the establishment of some infections. The effect of shading before inoculation seems to be qualitatively the same as that of darkness, a longer period of shading producing similar results to a short period of darkness, and the effects of darkness cannot be added to that of shading. This is shown in Table 2, which gives the results of a comprehensive experiment comparing various combinations of treatments of bean plants before and after inoculation with the tobacco necrosis virus. It will be seen that double shade increased susceptibility more than single shade, but that six days in double shade were slightly less effective than one day in the dark and that the effect on susceptibility of a period in the dark was not enhanced but slightly reduced, by previous shading.

The increased susceptibility of bean plants to the tobacco necrosis virus occasioned by a period of continuous darkness, persists for some time after the plants are returned to normal glasshouse conditions. This persistence of the effect of the treatment is shown in Table 3, where the results are given of an experiment in which

plants were kept in the dark for 24 or 48 hr. periods at different intervals during the 96 hr. before the time of inoculation. It will be seen that all the plants that had been in the dark produced more lesions than the controls, and that the largest number was produced by those that had spent the first 48 hr. in the dark and the second 48 hr. in the light. In other experiments this result was not precisely repeated, the effect of treatment usually decreasing with the length of time the plants spent in the light

TABLE 2. *The effect of shade and darkness on the susceptibility of bean plants to a tobacco necrosis virus*

Treatment of plants		Average no. of lesions per half-leaf with inoculum at	
Before inoculation	After inoculation	10 mg./l.	1 mg./l.
Light	Light	49	24
Light	48 hr. dark	31	16
Single shade 6 days	Light	146	61
Single shade 6 days	48 hr. dark	129	51
Double shade 6 days	Light	175	78
Dark 48 hr.	Light	192	88
Dark 24 hr.	Light	226	104
Single shade 4 days plus dark 48 hr.	Light	135	57
Single shade 5 days plus dark 24 hr.	Light	197	110

TABLE 3. *The effect of darkness at different times before inoculation on the susceptibility of beans to a tobacco necrosis virus*

Treatment of plants during the 96 hr. before inoculation	Average no. of lesions per half-leaf with inoculum at	
	10 mg./l.	1 mg./l.
Light 96 hr.	39	5
Light 72 hr., then dark 24 hr.	126	63
Light 48 hr., then dark 48 hr.	115	55
Light 48 hr., dark 24 hr., light 24 hr.	61	15
Light 24 hr., dark 48 hr., light 24 hr.	68	33
Dark 48 hr., light 48 hr.	154	67

Until 96 hr. before inoculation all the plants were kept in the light and they were all kept in the light after inoculation.

after removal from the dark cages. It may be that the plants used for this particular treatment were an unusually susceptible sample, a possibility inseparable from experiments in which treatments have to be applied to whole plants, or that at the time of starting the experiments they were in a specially suitable condition to respond to the period of darkness.

At the stage of development at which the bean plants were inoculated, their susceptibility to infection decreases so rapidly with increase in age that uneven germination of the seeds could cause appreciable differences between individual plants of one batch. For example, in one experiment two lots of bean plants, sown respectively on 24 and 27 June, were subjected simultaneously to the same

treatments before inoculation on 12 July. With inoculum at 10 and 1 mg./l. the younger plants produced an average of thirty-seven and eight lesions per half-leaf on the untreated plants and eighty and twenty-six on those kept for 24 hr. in the dark before inoculation, whereas on the plants 3 days older the average number of lesions per half-leaf was only seven and one on the controls and fifteen and five on the treated plants. Similar differences were obtained with the two batches of plants subjected to other treatments, the younger plants on the average producing between six and seven times as many lesions as the older ones at both dilutions of inoculum. In some experiments of this type, older plants have shown a greater increase in susceptibility after 48 hr. in the dark than after 24 hr., whereas the younger plants show the greater response to the 24 hr. treatment. This suggests that in the older plants the effect of darkness in increasing susceptibility may show over a longer period because they are not as sensitive as are younger plants to the deleterious effects of prolonged treatment.

Four experiments were made with the tobacco necrosis virus in tobacco plants, and these are summarized in Table 4. The increase in the number of lesions caused

Table 4. *The influence of various periods of darkness on the susceptibility of tobacco plants to a tobacco necrosis virus*

	Time spent by plants in the dark (hr.)							
	Before inoculation						After inoculation	
Exp. no.	120	96	72	48	24	0	24	48
1	—	—	177	238	140	40	36	19
	—	—	81	83	13	4	5	3
2	—	—	—	80	14	4	1	2
	—	—	—	13	5	1	0	0
3	192	113	88	32	23	11	—	—
	115	76	59	17	12	3	—	—
4	—	—	—	15	8	3	—	—
	—	—	—	7	1	1	—	—

In each experiment inoculum was used at 100 and 10 mg. virus per l., and the upper and lower row of figures gives the average number of lesions per half-leaf produced by the two concentrations. Except for the specified periods in the dark, either immediately before or immediately after inoculation, the plants were in normal glasshouse conditions.

by 24 hr. in the dark before inoculation was rather less than with this virus in beans, but with tobacco plants the susceptibility tended to increase over a much longer period of treatment than with beans. Putting the plants in the dark after inoculation again did not increase the numbers of lesions.

Tomato aucuba mosaic virus

Table 5 gives the summarized results of eight separate experiments made with tomato aucuba mosaic virus on tobacco plants and shows essentially the same effects as with the tobacco necrosis virus on this host. There were considerable differences

in susceptibility between batches of plants used in different experiments, shown by the differences between the numbers of lesions on the control plants produced by the same inoculum, but in all experiments a period in the dark before inoculation increased the numbers of lesion, whereas one after inoculation had little effect and usually reduced the number. The response to short periods in the dark was much less than that of beans to tobacco necrosis virus, but susceptibility increased much more with increasing length of time spent in the dark. In both host plants, however, it seems that changes in the plant during the period spent in the dark are of two kinds

TABLE 5. *The effect of periods of darkness on the susceptibility of tobacco plants to infection with tomato aucuba mosaic virus*

	Time spent by plants in the dark (hr.)							
	Before inoculation						After inoculation	
Exp. no.	120	96	72	48	24	0	48	72
1	—	—	—	212	136	97	118	104
	—	—	—	152	94	50	60	58
2	—	—	—	91	70	36	19	—
	—	—	—	14	8	5	2	—
3	—	—	—	132	32	82	85	70
	—	—	—	39	9	14	9	7
4	410	207	326	89	51	71	—	—
	171	64	104	17	7	15	—	—
5	—	—	—	91	70	36	19	—
	—	—	—	14	8	6	3	—
6	—	84	—	54	—	10	—	10
	—	34	—	22	—	5	—	6
7	—	68	—	40	—	20	18	—
	—	17	—	12	—	6	8	—
8	302	—	—	74	—	42	—	37
	181	—	—	27	—	11	—	13

In each experiment inoculum was used at 10 and 1 mg. virus per l., and the upper and lower row of figures gives the average number of lesions per half-leaf produced by the two concentrations. Except for the specified periods in darkness either immediately before or immediately after inoculation, all the plants were kept in normal glasshouse conditions.

in their effect on susceptibility to infection. The first kind shows predominantly by an increase in susceptibility as indicated by the increased numbers of lesions produced, and the second by a decrease, but whereas the second change begins to show itself in 24–48 hr. with beans it takes more than 5 days with tobacco plants at the stage of development used in these tests. Table 5 shows pre-inoculation treatments up to 120 hr., and during this time there is a continuing increase in susceptibility. In Exp. 8, however, an additional treatment of 144 hr. was given and this reduced the average number of lesions per half-leaf with the two dilutions of inoculum from 302 and 181 produced on the plants treated for 120 hr. to 172 and 74. The decrease in susceptibility produced by prolonged exposure to darkness with the different hosts

corresponds with the deleterious effects obvious to the eye, and it is probable that it coincides with degenerative changes in the host cells that lead to injuries too great to allow the development of obligate parasites such as viruses.

No experiments were made with tobacco plants to test the effects of complete darkness subsequent to shading, but in some there were comparisons of the relative effects of reducing illumination by various amounts. The maximum period of pre-inoculation treatment given was 9 days, and up to this time double shade gave a greater increase in susceptibility than single shade. The number of lesions produced by plants kept for 9 days in double shade was about 50% greater than the number produced by those kept in single shade, and was approximately equal to the number produced on plants kept for 3 days in the dark. Putting plants in the shade after inoculation had no consistent effects on the numbers of lesions produced.

With aucuba mosaic virus in tobacco, the character of the lesions, in addition to the susceptibility, was sometimes affected by the pre-inoculation treatment of the plants. The lesions produced on plants from the dark cages were black and sharply delimited from the neighbouring, uninfected, yellowish leaf tissue, whereas the leaves on plants kept in the light were brown and tended to merge diffusely into the green tissue.

Tobacco mosaic and tomato bushy stunt viruses

Only six experiments were made with *N. glutinosa*, three each with tobacco mosaic and tomato bushy stunt viruses, but there were no results to suggest that the susceptibility of this host to the two viruses was affected by shade or darkness in any manner qualitatively different from bean and tobacco plants to tobacco necrosis and tomato aucuba mosaic virus. Periods in single shade up to 12 days before inoculation increased the number of lesions produced by both viruses, as also did periods in the dark up to 3 days. Prolonging the period in the dark beyond this, however, led to obvious ill effects on the *N. glutinosa* plants and this was accompanied by a decrease in susceptibility. After slight shading, periods of 24 or 48 hr. in the dark increased the number of lesions, but after 10 days or more in single or double shade, 48 hr. in the dark usually caused a reduction.

Discussion

Little or nothing is known of the mechanisms involved in the establishment of virus infections, so that any interpretations of our results must be highly speculative, but some conclusions seem justified. With all the four viruses used, treatments of the host plants before inoculation that could be expected to reduce the amounts of photosynthetic products in the leaves also predisposed the leaves to infection, and, over a limited range, the increase in susceptibility was roughly proportional to the amount by which photosynthesis was reduced. This may not apply to all viruses, but it is probably true of many others. It could explain the observations by Samuel, Best

& Bald (1935) that plants reacted more uniformly and produced more lesions with tobacco mosaic and tomato spotted wilt viruses when kept for a day or so in the laboratory before inoculation than if kept continuously in the glasshouse. However, there may be additional factors involved with spotted wilt virus, for Best (1935) found that tobacco plants also produced more lesions if left in the laboratory for 2 or 3 days after inoculation than if they were kept in the glasshouse. With tobacco mosaic virus on *N. glutinosa*, on the other hand, fewer lesions developed on plants kept in the laboratory after inoculation than on plants kept in the glasshouse. This agrees with our results, in which shading plants or keeping them in the dark after inoculation with any of the four viruses did not increase susceptibility but often reduced it.

The different effects of similar treatments applied before and after inoculation suggest two distinct stages in establishing infection, the first being aided by a weakening of the cells and the second not. There is evidence from other sources for two such stages. For example, Kalmus & Kassanis (1944) found that treating leaves with atmospheres containing 40–60% CO_2 either immediately before or immediately after inoculation reduces susceptibility, whereas it was without effect if applied more than 4 hr. after inoculation. The two stages probably consist of (1) the entry of virus particles into cells and their attachment to essential sites for multiplication, and (2) multiplication of virus ultimately leading to the development of symptoms. Our results are probably concerned mainly with effects influencing stage (1), but they also suggest what might, perhaps, be expected with obligate parasites, that actual multiplication of virus particles proceeds better in well-nourished cells than in those with a reduced supply of metabolites.

A number of substances are known that act as inhibitors of plant viruses and prevent infection if they are present in the inoculum or are applied to the leaves before the viruses are inoculated. How such inhibitors produce their effects is uncertain, but it is known that they combine with the viruses and inhibitors are known to occur in the sap of some plants (Bawden, 1943). Their existence offers one seemingly obvious explanation for the increased susceptibility of host plants produced by pre-inoculation treatments that reduce or stop photosynthesis. It is possible that some of the labile products of photosynthesis are able to combine with the virus particles and render them non-infective; if this is so, then shading would be expected to prevent the accumulation of such substances, and a period in the dark would be expected to eliminate them from cells in which they had accumulated because of a prolonged period of photosynthesis at a high rate. Attempts we have made to test this explanation, by comparing the inhibitory power of sap from plants grown in normal glasshouse conditions with that of sap from plants kept in the dark, have failed to support it. When mixed with tobacco mosaic, tobacco necrosis and aucuba mosaic viruses, sap at various dilutions from bean and tobacco plants kept in the dark for periods up to 96 hr. all reduced infectivity to the same extent as sap from comparable plants kept continuously in normal glasshouse conditions. Such a negative result cannot, of course, disprove the explanation, for inhibitors could occur in

leaves but not be extracted in the sap, and there may be substances that are capable of combining with and neutralizing viruses *in vivo* but not *in vitro*.

In the absence of any positive evidence for the production during photosynthesis of any substances that act as virus inhibitors, however, it is reasonable to suspect a less specific and, perhaps, more mechanical explanation. The treatments in the dark before inoculation that produced considerable increases in susceptibility had no obvious effects on the fragility of the leaves, and it seems unlikely that they acted merely by making the epidermis more susceptible to wounding. On the other hand, they were sufficient to remove almost all the soluble carbohydrate; by affecting the turgor of the cells, this might facilitate the entry of virus particles, or it might act merely by reducing the cell components that interfere with the establishment of particles at multiplication sites. Much more work will be needed before any one of these possibilities can be preferred to any other, but it is clear that the physiological condition of the host is vitally concerned in determining susceptibility, and that conditions that predispose plants to infection are not necessarily those most favourable for virus multiplication.

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A STUDY OF THE LONGEVITY OF THE SEED-BORNE PARASITES OF FLAX IN RELATION TO THE STORAGE OF THE SEED

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(With 1 Text-figure)

From 1939 to 1947 observations were made on the longevity of *Colletotrichum linicola* Pethybr. & Laff., *Polyspora Lini* Laff., *Botrytis cinerea* Fr., and *Phoma* sp. occurring to different extents as seed-borne parasites of naturally contaminated samples of flax seed held under ordinary conditions of storage. The results obtained, together with the germination capacity of the seed estimated on different occasions during storage, are given and show that the longevity of the fungi considered varies within the following limits: *Colletotrichum linicola*, 26–69 months, *Polyspora Lini*, 16–55 months, *Botrytis cinerea*, 16–40 months and *Phoma* sp. 27–43 months. The data provide grounds for suggesting that the higher the percentage number of seeds contaminated with *Polyspora Lini* the longer will be the time taken for the death of the parasite to occur. As the time taken for the deactivation of the parasites is normally much longer than that for which flax seed for sowing purposes can be safely stored, prolonged storage of the seed does not commend itself as a likely method for securing the control of the diseases caused by seed-borne fungi. The germination capacity of the seed was normally found to be seriously impaired by the time all the parasites had died.

The possibility of controlling flax diseases caused by seed-borne fungi by relying upon the fact that such parasites tend to die out in seed stored for a comparatively long period has been referred to by several workers. Providing the necessary period of storage is not so long as to impair the germination capacity of the seed, such a method would merit serious consideration. Lafferty (1921), working in Ireland, found that when dry flax seeds contaminated with *Polyspora Lini* Laff. were stored, the conidia of the fungus retained their viability for $2\frac{1}{2}$ years after harvest, but he also found that the germination capacity of the seed had been impaired. On the other hand, Gentner (1923), working in Germany, suggested that flax seed could be used for sowing after storage for 5 years, by which time such seed-borne parasites as *Fusarium*, *Phoma* and *Botrytis* would have lost their viability. The present study has been made in order to obtain more exact information upon the longevity of seed-borne parasites in stored flax seed. It was commenced in 1939; the observations have been made over a period of 8 years and the organisms studied were *Colletotrichum linicola* Pethybr. & Laff., *Polyspora Lini* Laff., *Botrytis cinerea* Fr., and *Phoma* sp.

Materials

Twenty-one samples of flax seed contaminated to varying extents with the fungi under consideration were studied. The work with *Colletotrichum linicola* and *Polyspora Lini* was commenced in 1939, while *Botrytis cinerea* and *Phoma* sp. were not included in the investigation until 1941 and 1942 respectively.

METHODS

The seed samples were selected on the basis of their contamination, each being, as a rule, heavily contaminated with only one parasite. A sample consisted of 1 cwt. of dried seed with a moisture content of 10% or less which, after thorough mixing, was stored in an unheated room in the basement of the Agriculture Building. Small bulks of seed were drawn at random from each sample at intervals of about 2 months, and 500 seeds selected at random from each of these small bulks were examined by the Ulster method (Muskett & Malone, 1941) for the presence of seed-borne parasites. The first observation was usually made between 7 and 9 months after the harvesting of the crop. The examination of a sample was discontinued when it was found on three successive occasions that the fungus under observation was no longer viable. The longevity of a parasite was reckoned as the number of months elapsing between harvest and the time of making the first of the three successive examinations which yielded negative results.

Tests for the germination capacity of the seed were made concurrently with those for the presence of viable parasites. This work was carried out at the Seed Testing Station using the method whereby the seeds are placed on unglazed porcelain dishes firmly embedded on sand in Rodewald germinators. Either 300 or 400 seeds were used for each test which extended over a period of 10 days.

The relevant details of the seed samples, together with their percentage contamination with seed-borne parasites at the time of commencing the experimental work, are given in Table 1.

TABLE 1. *Relevant details of seed samples used*

Sample	Variety	Country of origin	Year of growth	Percentage no. of seeds contaminated with			
				C. linicola	*P. Lini*	*B. cinerea*	*Phoma* sp.
A	Stormont Gossamer	Denmark	1938	5·2	17·2	—	—
B	Stormont Gossamer	Denmark	1938	2·0	14·8	—	—
C	Liral Prince	N. Ireland	1938	19·6	5·0	—	—
D	Liral Prince	N. Ireland	1939	27·0	1·3	—	—
E	Blue Star	England	1939	0	23·7	—	—
F	Stormont Gossamer	Denmark	1939	35·7	0	—	—
G	Stormont Gossamer	N. Ireland	1940	0·4	50·4	—	—
H	Liral Monarch	N. Ireland	1940	14·6	1·6	—	—
J	Liral Dominion	N. Ireland	1941	31·2	1·6	—	—
K	Liral Crown	N. Ireland	1941	6·3	18·8	—	—
L	Liral Prince	N. Ireland	1941	0·6	0	16·2	0
N	Liral Prince	N. Ireland	1942	1·8	0·8	11·2	0
O	Liral Monarch	N. Ireland	1942	9·8	0·8	0	28·8
P	Liral Prince	N. Ireland	1942	35·2	0·8	0·5	1·5
Q	Stormont Gossamer	N. Ireland	1942	15·0	9·2	2·5	0·3
R	Liral Prince	N. Ireland	1943	0·6	0·6	11·6	1·8
S	Liral Prince	N. Ireland	1943	5·4	2·2	6·8	35·8
T	Stormont Gossamer	N. Ireland	1943	16·8	6·2	1·2	17·0
U	Liral Prince	N. Ireland	1943	19·0	0·8	0·4	10·4
V	Liral Prince	N. Ireland	1944	2·6	0	27·4	1·8
W	Liral Crown	Scotland	1944	6·8	0	8·6	50·0

Results

The results obtained for a number of seed samples contaminated with each of the fungi studied are presented graphically in Fig. 1 *a–d*. Since all the results obtained are not shown in this way the longevity of the parasites in each sample is stated in Table 2. Observations on samples K and P were discontinued before work with them had been completed owing to their having been damaged, while *Colletotrichum linicola* was still viable in sample W at the time of writing this account. Generally speaking, the longevity of the organisms varied within the following limits: *C. linicola*, 26–69 months; *Polyspora Lini*, 16–55 months; *Botrytis cinerea*, 16–40 months; and *Phoma* sp. 27–43 months.

Table 2. *Longevity of the seed-borne parasites of flax seed*

	Longevity (in months) of the parasite			
Sample	*C. linicola*	*P. Lini*	*B. cinerea*	*Phoma* sp.
A	55	55	—	—
B	42	44	—	—
C	39	29	—	—
D	43	27	—	—
E	—	31	—	—
F	69	—	—	—
G	43	53	—	—
H	29	17	—	—
J	50	40	—	—
K	Discontinued	Discontinued	—	—
L	40	—	40	—
N	43	33	37	—
O	43	29	—	43
P	Discontinued	19	Discontinued	27
Q	47	41	25	39
R	26	24	26	28
S	34	24	34	36
T	34	26	16	34
U	32	16	34	32
V	35	—	27	29
W	Not dead	—	27	27

The longevity of each of the four fungi has been correlated with the degree of contamination of the seed sample at the time of making the first examination which can be taken to approximate very closely to the degree of contamination at the time of harvest. The values of the correlation coefficients obtained are as follows:

Colletotrichum linicola	$r = +0{\cdot}4635$	($n = 15$, 5% point $= 0{\cdot}4821$)
Polyspora Lini	$r = +0{\cdot}6845$	($n = 14$, 1% point $= 0{\cdot}6226$)
Botrytis cinerea	$r = +0{\cdot}2467$	($n = 7$, 10% point $= 0{\cdot}5822$)
Phoma sp.	$r = +0{\cdot}1355$	($n = 7$, 10% point $= 0{\cdot}5822$)

Although a positive value was obtained in each case, only that for *Polyspora Lini*

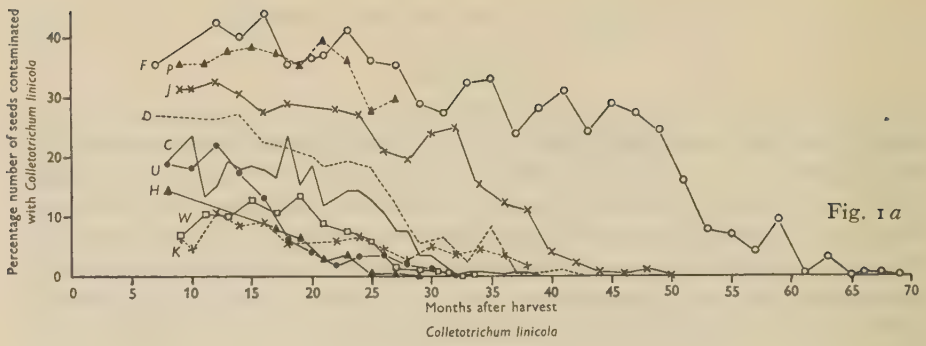

Fig. 1 *a*

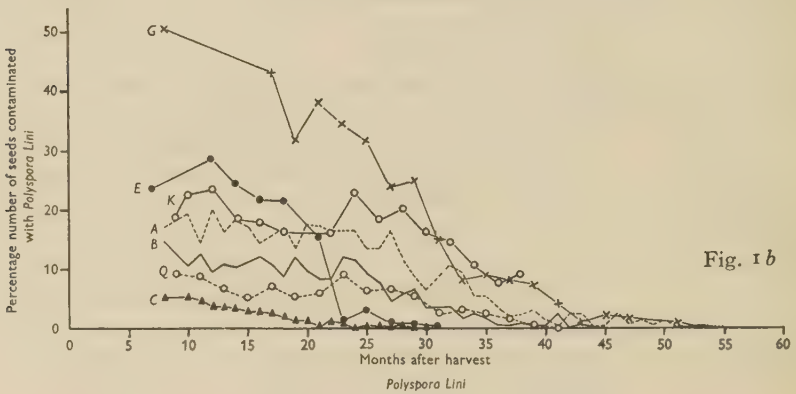

Fig. 1 *b*

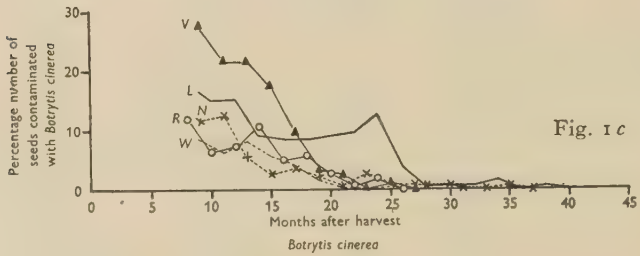

Fig. 1 *c*

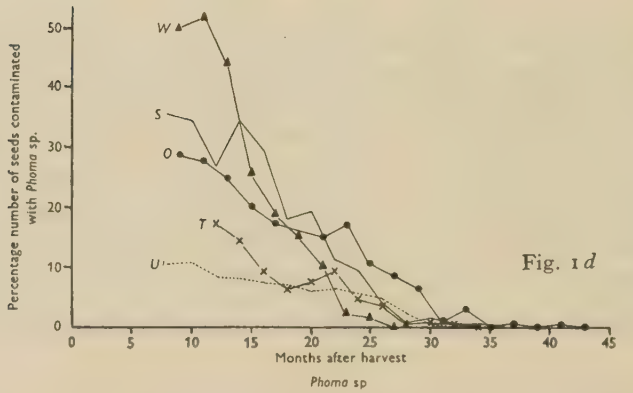

Fig. 1 *d*

Fig. 1 *a–d*. Longevity of the seed-borne parasites of flax seed. For key to lettering see Table 1.

can be regarded as significant, and the result obtained in this case suggests that, in general, the longevity of *P. Lini* is greater in heavily contaminated seed samples than in those which are but slightly affected.

The germination capacity of the seed at the time of threshing and when all the parasites had died out is shown for a number of seed samples in Table 3. From these results it is clear that the germination capacity of the seed had been seriously impaired by the time the parasites had become inactivated.

TABLE 3. *The effect of storage time on the germination capacity of flax seed*

Sample ...	F	J	N	O	Q	R	S	T	U	W
Germination on harvesting (%)	95	94	98	95	98	96	84	87	99	63
Germination after death of all parasites (%)	24	48	54	48	27	61	32	50	40	53

DISCUSSION

The employment of the Ulster method for the determination of the longevity of the seed-borne parasites of flax possesses the obvious advantage that the test establishes not only whether the spores which are present on the seed are viable but also if any mycelium which may be present is alive.

The longevity of the fungi in different samples showed substantial variation, but it was rarely found that any of the four organisms under consideration dies out earlier than 2 years after harvest. It may be suggested that the time taken for the death of any parasite to occur is related to the manner in which it is carried by the seed and this may explain why there is sometimes a noticeable difference in the times taken for a parasite to die out in samples contaminated by it to about the same extent. At the same time the data available suggest that in the case of *Polyspora Lini*, the more heavily the seed sample is contaminated, the longer will be the period required for the parasite to die.

In normal practice it is found that dry flax seed stored under good conditions will retain its germination capacity and energy in a satisfactory manner for a period of from 18 months to 2 years after harvest. It has also been shown (Davin, 1928) that the age of the seed may be of less importance than the conditions under which it has been stored. Although flax seed may be kept in good condition for sowing for long periods under ideal conditions of storage, such conditions do not normally apply in general practice where a period of 18 months is regarded as the maximum storage period desirable. The results obtained from this work show that, in general, the period required for seed storage in order that the seed-borne parasites present may become inactivated is much longer than that adopted in normal practice and that by the time the fungi have died out the germination capacity of the seed has become seriously impaired.

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SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

X. CONTROL OF THE DISEASE UNDER CONTINUOUS CULTIVATION OF A SPRING-SOWN CEREAL

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In a field experiment on the control of take-all at the Woburn Experimental Station, winter wheat was followed by two consecutive crops of spring-sown barley. Samples of the barley crop were taken from the forty-eight plots of the experiment in 1945 and 1946, for estimation of root Disease Rating, and grain yields were also recorded. A comparison of six autumn treatments of the stubble has shown that treatments affecting the available nitrogen content of the soil exercised a predominant effect upon incidence of take-all in the following crop. Two effects of nitrogen applied in autumn have been distinguished: (1) an immediate effect, in assisting survival of *Ophiobolus graminis* in infected root and stubble residues; (2) a deferred effect, in promoting disease escape of the following crop. The ploughing in of straw in autumn was found to increase the incidence of take-all, presumably because the adverse deferred effect of decomposing straw in locking up available nitrogen and withholding it from the following crop outweighed its beneficial immediate effect in helping to starve out *O. graminis*, by depriving the fungus of nitrogen. The autumn growth of undersown trefoil (*Medicago lupulina*) on the stubble land seemed to be entirely beneficial; active growth of the legume appeared to assist in starving out *O. graminis*, and nitrogen was released by decomposition of the trefoil in the soil after spring ploughing in time to benefit the barley crop immediately following.

From previous work, the chief requirement for rapid dying out of *Ophiobolus graminis* from infected stubble residues seems to be a shortage of available nitrogen (Garrett, 1944). The two most obvious ways of maintaining a condition of extreme nitrogen shortage in the soil after harvest appear to be: (1) incorporation of an easily decomposable nitrogen-poor organic material with the soil; (2) the growing of a catch-crop on the land immediately after harvest. The most practical way of applying the first measure is to plough in the long straw left by a combine harvester set at a high level. For the second alternative, the possibility of drilling or disking in a catch-crop of mustard or rape was first examined, but two serious potential difficulties became evident. First, successful establishment of a stand of mustard or rape depends upon the falling of rain not too long after harvest; secondly, the nitrogen content of the soil immediately after harvest may be actually too low for good establishment of the young catch-crop. Consideration was therefore given to a system for continuous barley growing empirically developed by Mr F. P. Chamberlain, of Crowmarsh

Battle Farm, Benson, Oxfordshire.* Mr Chamberlain sows with his barley a mixture of 10 lb./acre trefoil (*Medicago lupulina*) and 10 lb./acre Italian rye-grass. Some of the followers of his system, however, sow trefoil alone, at a seed-rate of 20 lb./acre, along with the barley. The trefoil, which makes a good growth in the autumn after harvest, is later ploughed into the soil as a preparation for the next barley crop.

The Chamberlain system appears, in theory, to give almost ideal conditions, so far as ordinary farming practice permits, for control of take-all under continuous cultivation of a susceptible spring-sown cereal. The active growth of the trefoil after harvest must keep free soil nitrogen at a low level (legumes growing actively under conditions favourable for carbon assimilation do not seem to excrete nitrogen into the soil but actually take it up; see Wilson, 1940). But when the trefoil is ploughed into the soil in late winter it releases nitrogen in the course of decomposition, which then helps the following barley crop to withstand any development of take-all that may occur. Indeed, Mr Chamberlain's success in controlling take-all and maintaining high barley yields by this system is probably due in part to his liberal use of artificial fertilizers as a supplement to the green manuring.

Design of the experiment

The experiment, of factorial design, comprised forty-eight plots (each $\frac{1}{50}$ acre), eight plots being allocated to each of six autumn treatments of the stubble. Of these eight plots assigned to each autumn treatment, four received 0·4 cwt. N/acre as sulphate of ammonia, shortly after sowing of the barley. Half the plots, again, received a combined dressing of 0·4 cwt. P_2O_5/acre as superphosphate and 0·5 cwt. K_2O as muriate of potash, also applied to the young barley. Lastly, half the plots were inoculated with a pure culture of *Ophiobolus graminis* on chaffed wheat straw at the beginning of the experiment. This $2 \times 2 \times 2$ treatment of the eight plots allotted to each autumn stubble treatment thus permitted no identical replicates.

The six autumn treatments were disposed in two groups of three each, early ploughed (September or October) and late ploughed (the following February). These treatments were as follows:

(1) Early ploughed. No other treatment.

(2) Early ploughed + 30 cwt. straw/acre, ploughed in.

(3) Early ploughed + 30 cwt. straw ploughed in with 0·4 cwt. N as sulphate of ammonia.

(4) Late ploughed, with stubble cleaning for control of weeds where necessary.

(5) Late ploughed, after autumn growth of trefoil sown with the barley in spring (at seed rate of 20 lb./acre).

(6) Late ploughed, after autumn growth of trefoil, with 0·4 cwt. N/acre as sulphate of ammonia, applied to the trefoil in autumn.

The experiment was thus designed to provide a comparison of the relative merits

* Our thanks are due to Mr Buddin, Mycologist for the South-Eastern Advisory Province, for directing our attention to the Chamberlain system.

of ploughed in straw and autumn growth of trefoil for control of take-all, in treatments (2) and (5), respectively. In treatments (3) and (6) nitrogen was deliberately added to the soil in autumn in an attempt to upset the postulated effect of straw and trefoil in reducing longevity of *O. graminis*. In treatments (1) and (4) a basic comparison was made between early and late ploughing of the stubble.

The experiment was carried out on Stackyard Field of the Woburn Experimental Station. The soil is a light loam; before the start of the experiment the whole area was given a dressing of carbonate of lime sufficient to bring soil reaction to pH 7. This site was chosen in preference to one on the Rothamsted Experimental Farm, because the light texture of the Woburn soil was known to be more favourable to the development of take-all than the heavier texture of the Rothamsted soil. Moreover, the fertility status of the area chosen for the experiment at Woburn was rather low, which was an additional factor favourable to the development of take-all. The experimental site had been cropped fairly frequently with cereals in the years preceding the experiment, and had become foul with *Agrostis* and *Holcus* spp., which are known to be troublesome propagators of *Ophiobolus graminis*. These grass weeds were eradicated by a full summer fallow immediately before the start of the experiment, but it was expected that natural inoculum of *O. graminis* would be well distributed over the whole area in consequence of this weed infestation.

After this summer fallow, Red Standard wheat was drilled over the whole experimental area on 25–7 September 1943; the appropriate plot received the combined dressing of superphosphate and potash on 11 November. On 6 December, the six alternate rows in twenty-four of the forty-eight plots were inoculated; a pinch (approx. 8 g.) of a pure culture of *O. graminis* on moist chaffed wheat straw was buried 1½–3 in. deep at 1-pace intervals. On 31 March 1944, this inoculation was found to have been successful at all sites examined. On 5 April, appropriate plots received sulphate of ammonia, and on 15 May trefoil was undersown in the wheat on all the predetermined plots. In the autumn of 1944, following harvest of the wheat, the six autumn treatments were carried out according to plan, and Plumage Archer barley was drilled on 2 March 1945; trefoil was sown on the appropriate plots at the same time. The barley was cut on 10–11 August, and stubble samples for disease estimation were taken on 15 August. The autumn treatments carried out in 1944 were repeated on the same plots in the autumn of 1945, and Plumage Archer barley was drilled again over the whole area on 19 March 1946, and the trefoil sown on the same day. In this year, samples for disease estimation were taken from the standing crop on 9 July, approximately 1½ months before the barley was cut (23 August). Estimates of the incidence of take-all were thus obtained, together with the grain yields of all the plots, for the two barley crops of 1945 and 1946.

Dates of other operations, not given above, are listed below:

Early-ploughed plots ploughed: 21–2 September 1944; 9–16 October 1945.

Autumn nitrogen applied to appropriate straw and trefoil plots: 25 September 1944; 1 November 1945.

Late-ploughed plots harrowed (as necessary for weed control): 4 December 1944; 5 and 16 October and 19 November 1945.

Whole area ploughed for barley (first ploughing for late-ploughed plots; second ploughing for early-ploughed plots): 24–6 February 1945; 19–20 February 1946.

Whole area harrowed before drilling barley: 1 March 1945; 19 March 1946.

Manures applied to barley: 9 April 1945; 10–11 April 1946.

Estimation of disease

Method of sampling. In 1945 stubble samples were taken shortly after the plots had been cut with the binder, and whilst the stooks were still standing, on 15 August. The plots were approx. 28 yd. in length; ten 8 in. samples of drill row were taken from each plot at 2-pace intervals. The initial sample was taken at 1–6 paces into the plot, and from any one of the twelve inside rows, both the row and the number of paces into the plot being determined by means of a table of random numbers. The remaining nine samples were taken at intervals of 2 paces forward and one row to the right, until the last row but one on the right had been sampled, after which the next sample was taken from the last row but one on the left side of the plot, and then the staggered progression to the right was continued.

In 1946 samples were taken from the standing crop on 9 July. The size of sample was increased from 8 in. (as taken in 1945) to 24 in.; each sample consisted of two 12 in. lengths, consecutive but in adjacent rows, and twelve samples were taken from each plot. Sites for sampling were determined wholly independently of one another (and not by the method used in 1945) by means of a table of random numbers, with the one restriction that three samples had to be taken from each quarter of the plot.

Disease rating of samples. Immediately after collection, the samples were dried for storage. After the roots had been washed free of soil, samples were separated into individual plants, which were classified by inspection into one of the following three categories:

Class I	Infection light or none	0 marks
Class II	Infection medium	1 mark
Class III	Infection severe	2 marks

The percentage disease rating for each individual sample was calculated as follows:

$$\frac{\text{Sum of numerical ratings of individual plants}}{\text{Number of plants} \times 2} \times 100.$$

The percentage disease rating for each plot was then calculated by taking the mean of the disease ratings for the ten or twelve individual samples.

Effect of treatments on disease rating

Before considering in detail the D.R.* values obtained from the 1945 and 1946 samples for disease estimation, which are set out in Tables 1 and 2, the effect of inoculation upon D.R. will be examined. In 1945 mean D.R. for all non-inoculated

* D.R. = disease rating.

plots was 26, and for all inoculated plots it was 31. In 1946 this comparatively negligible effect of inoculation upon D.R. was reversed; mean D.R. for all originally non-inoculated plots was 32, and for all originally inoculated plots it was 29. This unexpectedly slight effect of inoculation upon D.R. is to be attributed not to failure of the original inoculation (which was found to have been successful at all sites examined), but to the masking of it by a widespread natural occurrence of *O. graminis* throughout the soil of the experimental area. Multiplication of the fungus must have been favoured both by frequent cropping with cereals in the years preceding the experiment, and, more particularly, by a widespread infestation of the area by *Agrostis* and *Holcus* spp., which were eradicated by a summer fallow just before the start of the experiment. It is quite possible that the duration of the fallow was sufficient to kill the rhizomes of the grasses, but not to eradicate *Ophiobolus graminis* from the dead infected rhizomes; apparent instances of just such an occurrence have been seen elsewhere.

As the effect of inoculation upon D.R. has been so small, it has been possible to simplify presentation of the data by regarding inoculated and non-inoculated plots as replicates, and taking the mean D.R. for each pair (Tables 1 and 2). First may be

TABLE 1. *Percentage disease ratings for* 1945

	Early ploughing			Late ploughing			
	No addition	+straw	+straw +N	Plots harrowed	Trefoil	Trefoil +N	Means
No fertilizers to barley	55	59	41	36	28	18	39
PK only to barley	27	37	46	38	29	38	35
N only to barley	28	43	25	22	16	4	23
NPK to barley	17	22	12	31	3	12	16
Means (standard error ±3·3)	31	40	31	31	19	18	

TABLE 2. *Percentage disease ratings for* 1946

	Early ploughing			Late ploughing			
	No addition	+straw	+straw +N	Plots harrowed	Trefoil	Trefoil +N	Means
No fertilizers to barley	32	45	30	52	38	38	39
PK only to barley	35	37	19	36	29	41	33
N only to barley	36	36	30	28	20	13	27
NPK to barley	24	22	19	29	16	26	22
Means (standard error ±3·5)	32	35	24	36	25	29	

considered the effect of spring applications of nitrogen, and of phosphate and potash, upon D.R., as shown in the right-hand columns of Tables 1 and 2. The effect of nitrogen in reducing D.R. is highly significant ($P < 0{\cdot}001$), both in 1945 and in 1946. The effect of phosphate and potash in reducing D.R. is significant ($P < 0{\cdot}01$) in 1946, but not in 1945. These figures have already been quoted and discussed in the preceding paper of this series (Garrett, 1948); the effect of the fertilizers in reducing D.R. has been attributed to an increase in rate of crown-root production.

A proper appreciation of this important role of nitrogen, in particular, in promoting *disease escape* of the growing barley plant is necessary for full comprehension of the effects of autumn nitrogen, applied to the straw and trefoil respectively, upon the incidence of disease. Mean D.R.'s for the six autumn stubble treatments, given in the bottom rows of Tables 1 and 2, can be interpreted quite consistently on the assumption that two effects of nitrogen, both of which have already been established by experiment, are involved. First, there is an immediate effect of nitrogen applied in autumn in increasing survival of *O. graminis* in infected root and stubble residues. Secondly, there is a deferred effect of this nitrogen which is taken up by the decomposing straw and growing trefoil; that part of it which is released in the following spring (as an end-product in decomposition of the straw and trefoil residues) promotes disease escape of the growing barley crop.

Thus in 1945, the ploughing in of straw without nitrogen has increased D.R. from 31 to 40 (this effect just fails to reach significance at the 5% level); the beneficial effect of straw in locking up available nitrogen and helping to starve out the fungus has evidently been outweighed by its adverse effect in retaining this nitrogen after growth of the barley has started. Evidence of the second effect is provided by a reduction in grain yield from 1373 to 989 lb./acre (Table 3). Application of nitrogen along

Table 3. *Grain yields for* 1945 (*lb./acre*)

	Early ploughing			Late ploughing			
	No addition	+straw	+straw +N	Plots harrowed	Trefoil	Trefoil +N	Means
No fertilizers to barley	1106	768	700	1306	1181	1325	1064
PK only to barley	900	662	893	1225	1550	1556	1131
N only to barley	1862	1300	1293	1737	1768	1750	1618
NPK to barley	1625	1225	1788	1594	1850	2225	1718
Means (standard error ± 74)	1373	989	1168	1465	1587	1714	

with the straw has restored D.R. to the level of that for the no-straw plots, but has not fully restored grain yield. Trefoil, on the other hand, has evidently released its locked-up nitrogen through decomposition in time to benefit the barley crop; D.R. has been reduced from 31 in the late-ploughed, no trefoil plots to 19 (this reduction is significant at the 5% level), and grain yield increased from 1465 to 1587 lb./acre. The application of autumn nitrogen to the trefoil has not significantly affected D.R. This failure of autumn nitrogen here to reduce D.R. provides the chief evidence, so far as the results of this field experiment are concerned, that autumn nitrogen does in fact assist survival of *O. graminis*; did it not do so, then a reduction in D.R. due to the second, or deferred, effect of the nitrogen (in promoting disease escape of the barley) should certainly have been obtained. Evidence that the autumn nitrogen applied to the trefoil was actually taken up by the barley is provided by an increase in grain yield from 1587 to 1714 lb./acre.

Similar effects upon D.R. of the six autumn stubble treatments were obtained again

in 1946, as shown in Table 2, but one difference can be noted. In this, the second year of application, straw + autumn nitrogen has reduced D.R. from 32 in the no-straw plot to 24. This can be attributed, perhaps, to release of nitrogen applied with the straw in autumn 1944, and withheld by the straw from the 1945 crop, in time to benefit the 1946 crop (i.e. by promoting disease escape through increased crown-root production). This contention is supported by the figures for grain yield (Tables 3 and 4); in 1945 straw + autumn nitrogen reduced grain yield from 1373 in the no-straw plots to 1168 lb./acre, whereas in 1946 grain yield was actually increased, from 737 to 1128 lb./acre.

TABLE 4. *Grain yields for* 1946 (*lb./acre*)

	Early ploughing			Late ploughing			
	No addition	+straw	+straw +N	Plots harrowed	Trefoil	Trefoil +N	Means
No fertilizers to barley	501	525	982	384	1009	1163	761
PK only to barley	580	475	1097	650	1438	1763	1001
N only to barley	838	850	1201	1069	1294	1584	1139
NPK to barley	1032	984	1232	1338	1869	1863	1386
Means (standard error ± 56)	737	709	1128	860	1403	1593	

Evidence for these two contrasting effects of autumn nitrogen upon D.R. may now be briefly recapitulated as follows. The deferred effect of autumn nitrogen, released through eventual decomposition of straw or trefoil residues, in promoting disease escape of the growing barley crop through increase in crown-root production, can be postulated by reference to the observed effect of nitrogen applied in spring. Evidence of the converse of the deferred effect, i.e. of a withholding of soil nitrogen from the crop, is provided by the straw treatment. Evidence of an immediate effect of autumn nitrogen in assisting overwintering survival of *O. graminis* is provided by the trefoil plots, and can be inferred by the failure of autumn nitrogen to reduce D.R. In other words, on the trefoil plots the two effects of autumn nitrogen upon D.R. balance one another.

In the foregoing discussion, trends in grain yield have received only incidental consideration, in so far as they have been used to elucidate variations in D.R. A comparison of Tables 1 and 2 with Tables 3 and 4 shows that, almost without exception, treatments that reduced D.R. also increased grain yield. Only a proportion of this increase in grain yield can be attributed to the parallel decrease in incidence of disease, because the range of variation in grain yield is much wider than the range of variation in D.R. Variations in D.R. seem to have been caused chiefly by variations in available nitrogen content of the soil under the growing barley crop. On the type of soil on which this experiment has been carried out, spectacular responses in grain yield to applications of nitrogen are normally obtained, even in the absence of disease. In this respect, therefore, it is fortunate that measures for the control of take-all can be expected to give a substantial increase of yield in the absence, as well as in the presence, of the disease.

Lastly, there is the practical question as to which of the six autumn treatments is of greatest value for control of take-all and maintenance of grain yield. For this purpose, only the plots receiving spring nitrogen need be considered, as the relative performance of plots receiving no spring nitrogen is chiefly of academic interest. In Table 5 are given mean D.R.'s for the six autumn treatments, for all plots receiving

Table 5. *Percentage disease ratings for plots receiving spring dressing of nitrogen*

	Early ploughing			Late ploughing		
	No addition	+straw	+straw +N	Plots harrowed	Trefoil	Trefoil +N
1945	22	32	18	26	9	8
1946	30	29	24	28	18	19
Means	26	31	21	27	14	14

spring nitrogen. It is obvious from the Table that the two trefoil series have given the best control of take-all: they have also consistently given the highest yields of grain (Tables 3 and 4). It seems likely, however, that if the autumn nitrogen applied to the trefoil had been applied instead to the barley in spring, so as to have doubled the spring dressing, it would still further have lowered D.R. and increased yield.

Grateful acknowledgement is made to Dr D. J. Watson for the statistical design of the experiment.

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STUDIES IN PLANT NUTRITION

I. THE EFFECT OF SOME ORGANIC SUPPLEMENTS ON THE GROWTH OF LETTUCE IN SAND CULTURE

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(With 2 Text-figures)

The effect on growth and development of lettuce (May King Improved) resulting from the addition of organic supplements to a full inorganic culture solution has been investigated by means of sand cultures.

The addition of an aqueous extract of leaf mould resulted in enhanced growth on both a fresh and dry-weight basis. Shoot and particularly leaf growth were most markedly stimulated so that a higher shoot/root ratio was obtained. The plants receiving leaf-mould extract were also characterized by a slightly higher moisture content.

The addition of either yeast extract or hydrolysed casein did not result in any significant difference in growth as compared with plants receiving only inorganic nutrients.

The results are briefly discussed against the background of a review of the present status of our knowledge of the role of organic materials in the nutrition of higher green plants.

General introduction

Recent work on the growth requirements of fungi, bacteria and of the isolated root systems of higher plants has led to the recognition of essential growth substances of three main types: specific cell metabolites, components of enzyme systems (vitamins) and hormones (auxins). Heterotrophic organisms are either incapable of synthesizing certain of these growth substances or can synthesize them only so slowly that their omission from the medium causes reduction of growth and of metabolism to a sub-optimum level. It is possible that such considerations also apply to higher green plants and that some of these may differ in their synthetic abilities, and that at least certain species, even if they are able to grow indefinitely on a purely inorganic medium, may only achieve suboptimum development under these conditions. Furthermore, the synthetic ability of some plants may be modified, not only quantitatively, but also qualitatively, by environmental conditions so that the effect of available organic nutrients may be of particular significance in a suboptimum environment.

The experimental investigation of this question is fraught with certain difficulties. The use of sand or solution cultures to achieve an inorganic medium and to effect comparison with soil cultures involves both a change in the physical conditions around the roots and in the conditions of presentation of inorganic nutrients. When using sand or solution cultures to study the effect of organic materials on growth and on development it is necessary to take account of the possibilities that organic

supplements may simultaneously provide some nutrient element in which the inorganic culture solution is deficient and that the presence of organic material may affect the absorption of ions. It is, therefore, important that in such experiments the inorganic nutrient supply approximate as closely as possible to the optimum and that the fullest possible chemical analysis of the plant's tissues should be undertaken in evaluating the effects of organic supplements on plant development. It is doubtful if plants have been successfully grown to maturity in an inorganic medium under conditions excluding such obvious sources of organic material as dust and micro-organisms. Furthermore, initial reserves of essential growth substances in the seed or spore might only be effectively depleted if such inorganic nutrition was carried through several generations. To prove that, in fact, there is no positive response of specific higher plants to traces of organic material will necessitate the use of the widest possible range of organic supplements, the variation of environmental conditions, the passage through several life cycles and ultimately the achievement of rigorously inorganic cultures developing under sterile conditions.

From time to time various workers have claimed that the beneficial effects of organic manures are not fully explained either by their content of known nutrient elements or by the resulting improvement in the physical conditions of the soil (Hartley & Greenwood, 1933). Siddappa & Subrahmanyan (1934), submitting this hypothesis to experimental study, were unable to obtain any stimulation of growth by the application of minute quantities of the water-soluble constituents of organic manures to sand-cultured barley, though both Iyer, Siddappa & Subrahmanyan (1935) and Virtanen & Hausen (1934) observed a marked stimulation of the growth and of flowering of various plants to result from injection into the plant of small amounts of such extracts.

More recently, extracts of dung and of other substances have been found to promote increase in frond number in *Spirodela*, an effect which could not be accounted for by their known content of nutrient elements or of growth substances (Gorham, 1943). Cow-manure extract applied to *Cinchona* seedlings has also been found to give greener plants than the controls, an effect which could not be imitated by applications of nitrogen, auxins, amino-acids or a number of other growth substances (Harper & Cooper, 1943).

Bottomley (1914*a*) described the preparation of an extract of 'bacterized peat' which he claimed had a markedly stimulating effect on the growth of wheat and of other plants. The beneficial effect of this extract could not be accounted for on the basis of its content of nutrient elements (Bottomley, 1914*b*, *c*). Bottomley postulated that it contained organic growth-promoting substances for which, in 1915, he introduced the term 'auximones' and which he subsequently showed were apparently also present in well-rotted stable manure, leaf mould, yeast, root nodule tissue and a number of germinating seeds. More extensive experiments were undertaken from 1917 to 1920 (Bottomley, 1917, 1919, 1920*a*, *b*, *c*) with water plants, such as *Lemna minor*. It was concluded that 'auximones' were essential for the sustained growth

and reproduction of *Lemna*, that they were required only in small quantity and that an effect equal to that of 'bacterized peat' extract could be obtained by supplying nucleic acid derivatives, isolated from peat, and mixed with a culture of nitrogen-fixing bacteria. Confirmatory evidence was advanced by Mockeridge (1920, 1924*a*, *b*), who also emphasized that the 'auximones' were produced during the degradation of nucleic acid. The subsequent work of Clark & Roller (1924, 1930, 1931), Clark (1926), Saeger (1925), Wolfe (1926) and Ashby (1929) has shown that whilst *Lemna* can grow and multiply for prolonged periods in a purely inorganic medium, under carefully controlled conditions, the addition of catalytic amounts of organic matter to the cultures leads to a marked increase in growth rate. However, *Lemna* is a plant which in nature reaches optimum development in stagnant water rich in organic decomposition products, and it remains for future work to show how far 'auximones' are important for the optimum growth and development of typical land plants.

Schreiner and co-workers, at the Bureau of Soils of the U.S. Department of Agriculture, have isolated from fertile soils a wide variety of organic nitrogen compounds including creatinine (Schreiner, Shorey, Sullivan & Skinner, 1911), xanthine, hypoxanthine, adenine (Schreiner & Shorey, 1910; Lathrop, 1917) and cystine (Schreiner & Shorey, 1910). These nitrogen compounds were shown to be capable of replacing nitrate as the sole source of nitrogen during the growth of young wheat plants, but in no case was any distinct stimulation of growth observed when an adequate nitrate supply was present.

There is much evidence to show that non-leguminous plants can derive benefit from being grown in association with legumes (Thornton & Nicol, 1934; Nicol 1934). It is suggested that substances secreted into the soil by the leguminous roots are utilized by the associated crop. Virtanen and co-workers (Virtanen 1938, 1939; Virtanen & Laine, 1939; Virtanen, Linkola, Hakala & Rautanen, 1946) have obtained evidence that aspartic acid, together with smaller amounts of β-alanine, oximino succinic acid and glutamic acid, are excreted by roots bearing nodules. It is, therefore, possible that growth of the associated crop is enhanced by organic nitrogenous metabolites derived from the legumes.

Frank and Melin are reported (Rayner & Neilson-Jones, 1944) to consider that the formation of mycorrhiza is of significance in relation to the nitrogen supply of the higher plant and to suggest that this takes the form of readily available organic nitrogen compounds liberated by the fungal partner. If the estimate of Neilson-Jones that about 80% of flowering plants develop mycorrhiza should prove correct then serious doubt will be cast on the view that the inorganic soil nitrogen is of direct and primary significance in the nutrition of higher land plants.

McCarrison (1924, 1926), Nath & Suryanarayana (1927) and Rowlands & Wilkinson (1930) claim to have demonstrated a higher vitamin content in crops grown with organic as compared with inorganic fertilizers, and Nath & Suryanarayana have correlated this with higher seed quality as judged by cropping capacity in the next generation. More recently, however, Arnon, Simms & Morgan (1947) have

reported that the nutritive value of Astoria bent grass, when tested as the exclusive diet of guinea-pigs, is not enhanced by growth on soil treated with organic manure as compared with an inorganic medium. Various clinical investigations (Wendt, Pies, Prüfer, Barth, Schröder, Catel, Dost, Scheumert, Reiter & Ertel, 1938; Schuphan, Dost & Schotola 1940) have suggested that crop plants grown with inorganic fertilizers are inferior from the standpoint of human nutrition to those grown on humus rich soils, but the evidence at present available does not justify the sweeping conclusions reached by some authors (Friend, 1937; Daldy, 1940).

The demonstration of the presence of appreciable quantities of vitamin B_1 in a number of organic manures (Bonner & Greene, 1938) and of the importance of certain vitamins, particularly those of the B group, as essential growth factors for micro-organisms (Knight, 1945) and for isolated root cultures, (White, 1943) has led to a number of investigations into the effect of pure vitamins on the growth and the development of higher plants growing in an otherwise inorganic medium. Bonner & Greene (1938, 1939) have reported better root and shoot growth in a number of plant species when these were supplied with vitamin B_1. Börgstrom (1939) applying both vitamin B_1 and ascorbic acid, and Dennison (1940) using vitamin B_2, have obtained similar beneficial results. A number of workers have criticized these findings and in their own experiments have been unable to detect any increase in growth from application of pure vitamins to sand cultures of a number of different plants (Arnon, 1940; Hamner, 1940; Swartz 1941; Minnum, 1941; Templeman & Pollard, 1941). It is, therefore, not yet possible to claim that any land plants are limited in their growth by an inherently low rate of vitamin synthesis or to assess the significance of the vitamin content of organic manures in plant nutrition.

It has been shown that auxins are produced by many fungi and bacteria, and in 1935 Thimann brought forward evidence that the growth hormone formed by many of these micro-organisms is indole-acetic acid. Berezova, Naumova & Rasnizina (1938) suggested that the growth-stimulating activity of peat 'bacterized' with *Azotobacter* is due to the high production of growth hormones by the bacteria. This view is supported by the more recent work of Clark & Frahm (1940) and Gorham (1941), who have reported a stimulation of growth in *Lemna* under sterile conditions to result from treatment with indole-acetic acid. Roberts & Roberts (1939) have isolated growth-hormone-producing bacteria from soils and Parker-Rhodes (1940) has shown the normal presence of a heteroauxin-like substance in soils. The work of Stewart & Anderson (1942) is of particular interest. It reveals a suggestive parallel between growth-hormone content and fertility in a number of different soils. This raises the question of the significance for plant development of auxins present in the soil, or added in organic manures. Duhamet (1939) has shown that indole-acetic acid may stimulate the growth of excised roots in culture, and a number of workers (Amlong, 1936, Fiedler, 1936, Thimann, 1936; Kraus & Mitchell, 1939; Mitchell & Stewart, 1939; Hamner, 1941; Laude, 1941) have reported that synthetic

growth substances, when applied at certain concentrations, may stimulate root development of plants growing in sand or in soil. The increased root development, observed by Breazeale (1927) and Hilletzer (1932), as a result of the application of extracts of animal and of vegetable manures and of humus may be explicable on the basis of their auxin content. However, studies of the effect of auxins on general plant development have given conflicting results. While a number of workers (Amlong & Naundorf, 1937, 1938, 1939; Grace, 1937, 1938*a*, *b*; McRostie, Hopkins & Grace, 1938; Mitchell & Hamner, 1938; Thimann & Lane, 1938; McEvoy, 1939; Stier & DuBuy, 1939; Hwang & Pearse, 1940) have reported significant increases in dry weight resulting from applications of synthetic growth hormones, others (Pearse, 1936*a*, *b*; Templeman, 1939; Templeman & Marmoy, 1940; Hamner, 1942) have observed a decrease in growth rate. The observation of Stephenson (1943) that lettuce seedlings grown for 2 weeks under aseptic conditions show pronounced increases in the dry weight of both root and shoot as a result of treatment with naphthaleneacetic acid, at a concentration of 10 parts per billion, is of particular interest in the present investigation.

Previous work relating to the possible role of organic soil constituents in the development of higher plants directs attention to the possible importance of organic nitrogenous metabolites, vitamins and auxins. The evidence, however, fails to establish satisfactorily the necessity of an external supply of any of these substances for optimum development. The significance of organic soil constituents for the growth and the development of higher land plants appears to be a fruitful, and as yet little explored, aspect of the problem of soil fertility.

Experimental

Culture vessels

The culture vessels used were 48 × 7 in. diameter plant-pots covered internally with a double coat of 'Bituros' solution (Wailes Dove Bitumastic Ltd.) and with the drainage hole plugged with glass-wool. Each pot contained approximately 4 kg. of dry water-washed Garsides 2L silica sand. This quantity of sand retained approximately 850 ml. of water under conditions of free drainage.

Culture solution

The culture solution contained:

	mg./l.
Ammonium sulphate, $(NH_4)_2SO_4$	39·7
Magnesium sulphate, $MgSO_4.7H_2O$	51·8
Calcium nitrate, $Ca(NO_3)_2.4H_2O$	53·3
Sodium nitrate, $NaNO_3$	110·5
Potassium dihydrogen phosphate, KH_2PO_4	78·2
Disodium hydrogen phosphate, $Na_2HPO_4.12H_2O$	46·2

These quantities correspond to the following p.p.m. of the cultural elements: NO_3–N, 24·5; NH_4–N, 8·47; S, 16·44; P, 21·84; K, 22·44; Mg, 5·05; Ca, 9·03; Na, 36·0.

To each litre of this solution was added 1 ml. of a micro-nutrient solution which contained:

	g./l.		g./l.
Boric acid, H_3BO_3	0·6	Lithium chloride, $LiCl.2H_2O$	0·03
Manganese chloride, $MnCl_2.4H_2O$	0·4	Titanium dioxide, TiO_2	0·03
Zinc sulphate, $ZnSO_4.7H_2O$	0·09	Stannous chloride, $SnCl_2.2H_2O$	0·03
Copper sulphate, $CuSO_4.5H_2O$	0·05	Nickel sulphate, $NiSO_4.6H_2O$	0·05
Aluminium chloride, $Al_2Cl_6.12H_2O$	0·09	Ferric citrate, $FeC_6H_5O_7.3H_2O$	6·24
Potassium iodide, KI	0·03	Sodium silicate, Na_2SiO_3	0·43
Potassium bromide, KBr	0·03	Potassium permanganate, $KMnO_4$	0·37
Cobalt nitrate, $Co(NO_3)_2.6H_2O$	0·05		

The micro-nutrient elements were therefore present in the final culture solution to the following p.p.m.: B, 0·10; Mn, 0·24; Fe, 1·2; Zn, 0·02; I, 0·023; Br, 0·02; Si, 0·10; Cu, 0·013; Co, 0·01; Ti, 0·018; Sn, 0·016; Ni, 0·01; Al, 0·004; Li, 0·003.

The complete culture solution was prepared fresh each week by dilution of concentrates with City of Nottingham tap water.* The pH of the culture solution was 6·45–6·55.

Nutrient treatments

Four different nutrient treatments were employed, namely:

Treatment I. Complete culture solution + 10 ml./l. of a solution of sodium nitrate containing 6·65 mg. N/10 ml.

Treatment M. Complete culture solution + 10 ml./l. of an aqueous extract of leaf mould containing 6·65 mg. N/10 ml.

The leaf mould, obtained from a well-rotted heap of leaves (mainly oak), was passed through a $\frac{1}{4}$ in. mesh sieve and air dried. 200 g. of this prepared mould was treated with 800 ml. of water previously heated to 60° C. and the macerate stored in the refrigerator overnight. The free liquid was strained off and the solid residue expressed in a double-action tincture press. The combined liquids, after filtration through paper, were concentrated under reduced pressure at a temperature not exceeding 40° C. to give the aqueous extract of leaf mould. The extract was freshly prepared each week from a standardized bulk sample of mould and stored in the refrigerator.

Treatment C. Complete culture solution + 10 ml./l. of an aqueous solution of casein hydrolysate containing 6·65 mg. N/10 ml.

The aqueous solution of casein hydrolysate was prepared by dilution of a sterile solution of enzyme hydrolysed casein kindly presented by Genatosan Ltd., Loughborough. The aqueous solution was prepared in bulk and distributed into tubes containing 30 ml. each, which were subsequently stored at −20° C.

Treatment Y. Complete culture solution + 10 ml./l. of an aqueous solution of yeast extract containing 6·65 mg. N/10 ml.

* A typical analysis of this tap water gave the following data: pH 9·5; p.p.m.: total solids 100, chloride–Cl 14·2, free ammonia 0·02, albuminous ammonia 0·01, oxidized nitrogen as nitrate nil, as nitrite 0·005, temporary hardness 15·0, permanent hardness 50·0.

The aqueous solution of yeast extract was prepared by mixing 10 g. Yeastrel (Brewers' Food Supply Co. Ltd, Edinburgh) with 800 ml. water, filtering and adjusting to the required N content. The aqueous solution was stored as described for the aqueous solution of casein hydrolysate.

Experimental plan

There were twelve replicates of each of the four treatments. The pots were arranged on the bench of an unheated greenhouse as a layout of twelve randomized blocks (blocks 1–12).

The sand of each pot was saturated with the appropriate treatment solution at half strength on 1 May 1947. On 2 May each pot was set with eleven seeds of the lettuce variety May King Improved (Watkins and Simpson). The seeds were carefully spaced to give a central group of five seeds and a peripheral ring of six seeds. On 3, 6 and 8 May each pot received 250 ml. of the treatment solution at

Fig. 1. Specimen plants of the M, I, C and Y treatments photographed on 1 July 1947

half strength. From 9 May to 2 July each pot received, three times per week, and, according to weather conditions, either 250 ml. or 500 ml. of the full-strength treatment solution. From 4 to 15 July each pot received 500 ml. of the full-strength treatment solution three times a week. A free flow of drainage occurred at each application of the treatment solution, but in order to prevent any accumulation of salts the pots were flushed with 500 ml. of water on 4, 14 and 24 June, and on 3 July.

Germination was complete by 16 May. The seedlings had two expanded plumular leaves by 20 May, were in the six-leaved stage by 3 June and had eight to twelve leaves by 13 June. On 27 June all plants of the I, C and Y treatments were noticeably inferior to those of the M treatment in degree of 'hearting' and were showing signs of bolting. By 1 July all plants showed signs of bolting, but this was less advanced in those receiving M treatment (Fig. 1). By 4 July all plants were actively bolting and flower buds were visible. The height of any plant receiving the M treatment was now equal to, or greater than, that of any of the plants receiving the other three

treatments in each replicate block. By 9 July the plants receiving M treatment were higher and had more advanced inflorescences than those of the other treatments (Fig. 2). Throughout the experiment the plants remained healthy and quite free from tinting. A mild leaf scorch affecting the oldest leaves had developed in plants of the Y and C treatments by the time of the final harvest on 15 July.

Fig. 2. Specimen plants of the M, I, C and Y treatments photographed on 9 July 1947

The plants were harvested as follows:

No.	Harvest dates	Plants left per pot
	Seedling harvests	
1	20. v. 47	1 central, 4 peripheral
2	3. vi. 47	1 central, 2 peripheral
3	13. vi. 47	1 central
	Main harvests	
4	2. vii. 47	6 blocks left
5	15. vii. 47	Final harvest

At the seedling harvests the following data were recorded for each seedling:

Harvest 1—fresh and dry weights of whole seedling.

Harvest 2—fresh and dry weights of whole seedling, number of leaves and dimensions of largest leaf.

Harvest 3—fresh and dry weights of whole seedling and of shoot system, number of leaves and dimensions of largest leaf.

At the main harvests the following data were recorded for each plant:

Harvests 4 and 5—fresh and dry weights of whole plant, leaves, stem (including inflorescence), and roots; height of shoot system.

The dried tissues were stored in air-tight containers for subsequent analysis. The dry weights were determined by immediately drying the fresh tissues by the method previously described (Street, Kenyon & Watson, 1946).

Results

The main experimental data are summarized in Tables 1 and 2. The statistical analysis of the randomized block layout was carried out as described by Paterson (1939).

Table 1. *Mean fresh weights and dry weights for single plants and plant organs in grams*

F = fresh weights; D = dry weights; W = whole plant; S = whole shoot; L = leaves; St = stem (with inflorescence if present); R = roots.

Harvest no.	Description of data	Treatment means (g.) I	M	C	Y	Significant difference between means for $P=0.05$	$P=0.01$	Result
1	FW	0·165	0·190	0·187	0·191	F test negative		I=M=C=Y
	DW	0·009	0·010	0·010	0·011			
2	FW	2·73	3·54	2·76	2·96	0·27	0·37	M>I=C=Y
	DW	0·157	0·199	0·158	0·172	0·018	0·025	M>I=C=Y
3	FW	13·6	17·3	13·3	13·4	1·04	1·37	M>I=C=Y
	DW	0·98	1·2	0·97	0·98	0·12	0·16	M>I=C=Y
	FS	12·12	15·4	11·9	11·9	1·40	1·88	M>I=C=Y
	DS	0·85	1·01	0·83	0·84	0·08	0·11	M>I=C=Y
4	FW	87·9	115·0	90·7	83·3	11·5	17·1	M>I=C=Y
	DW	6·21	7·51	7·13	5·95	1·05	1·45	M=C.C=I M>I=Y.C>Y
	FL	45·4	65·8	45·5	44·7	7·3	10·1	M>I=C=Y
	DL	2·93	4·31	3·15	3·0	0·89	1·23	M>I=C=Y
	FSt	15·4	17·2	15·7	13·9	F test negative		I=M=C=Y
	DSt	1·25	1·11	1·39	1·01	F test negative		I=M=C=Y
	FR	25·9	31·9	29·5	24·6	4·5	6·2	M>I=Y.M=C C>Y.C=I
	DR	2·01	2·10	2·60	1·94	0·40	0·56	C>M=I=Y
5	FW	122·5	162·6	114·4	120·6	13·5	18·6	M>I=C=Y
	DW	12·27	14·88	11·52	11·81	0·81	1·12	M>I=C=Y
	FL	51·7	71·2	46·4	51·9	3·9	5·3	M>I=Y>C
	DL	4·34	5·31	3·80	4·07	0·56	0·78	M>I=C=Y
	FSt	39·9	56·8	37·2	37·5	8·6	11·9	M>I=C=Y
	DSt	5·17	6·53	4·83	4·81	0·80	1·11	M>I=C=Y
	FR	30·9	34·6	30·7	31·2	F test negative		I=M=C=Y
	DR	2·76	3·03	2·91	2·93	F test negative		I=M=C=Y

TABLE 2. *Mean moisture contents (percentage of fresh weight) for plants and plant organs*

Harvest no.	Description of data	Treatment means I	M	C	Y	Significant difference between means for $P=0.05$	$P=0.01$	Result
2	W	94·0	94·4	94·1	94·25	F test negative		I=M=C=Y
3	W	92·5	92·9	92·6	92·7	F test negative		I=M=C=Y
	S	92·9	93·4	93·0	92·9	F test negative		I=M=C=Y
4	L	93·5	93·4	93·1	93·3	F test negative		I=M=C=Y
	St	92·0	93·5	91·2	92·6	1·4	1·9	M>I=C.M=Y
	R	92·2	93·4	91·2	92·3	1·0	1·3	M>I=Y>C
5	L	91·8	92·6	91·8	92·2	F test negative		I=M=C=Y
	St	87·1	88·6	87·0	87·3	0·9	1·2	M>I=C=Y
	R	91·0	91·1	90·5	90·6	F test negative		I=M=C=Y

No significant difference between treatments was revealed by the data on the number of leaves and dimensions of the largest leaf recorded for harvests 2 and 3, or in the height of the shoot recorded for harvest 4. The treatment means in centimetres for the height of the shoot at harvest 5 were M = 65·2, I = 60·2, C = 58·0, Y = 58·5, with a significant difference between means for $P=0.01$ of 3·8, so that M > I = C = Y. (M significantly greater than other treatments, other treatments not significantly different from one another).

The number of seeds set under each treatment was 132, the percentage germination for each treatment was M = 82·6; I = 81·8; C = 74·2; Y = 74·4.

DISCUSSION

Woodman has studied the phosphorus (1936, 1939*a*), nitrogen (1939*b*), potassium (1939*c*, *d*), calcium and magnesium (1940*a*) requirements of May King lettuce grown in sand culture and has devised a nutrient solution (1940*b*) of optimum concentration. The culture solution used in this investigation is based on that of Woodman. The existence of this data, together with the fairly short growth period and the suitability for greenhouse culture determined the choice of lettuce as one of the plants to be used in our initial experiments.

Low temperature during germination and early growth (Gray, 1942; Milthorpe & Horowitz, 1943; Simpson, 1943), particularly if followed by high temperature and long photoperiods, stimulates seed-stalk production in lettuce. The long photoperiods (Rudorf & Stelzner, 1934; Reimers, 1939) and high temperatures during later growth (Bewley, 1935; Thompson, 1940) stimulate 'bolting' particularly in forcing and winter varieties. The variety May King is a medium short-day early summer or forcing lettuce. Plants from seed set in May would therefore be expected to 'bolt' early and complete their life cycle in the shortest time. This is confirmed by data just published by Woodman & Johnson (1947), which show that plants from

seed set in the period May–July 'bolted' in the shortest time, did not 'heart', were of small weight at the time of 'bolting' and were characterized by high top/root ratios and high percentages of moisture in the 'tops'. The choice of a setting date in May for our initial work on lettuce was made to study the treatment effects on growth and development under conditions favouring inflorescence development. The carrying out of a similar set of cultures from seed sown in the period September–November is regarded as a necessary part of future work.

The data obtained for the percentage germination under each treatment are to be accepted with caution in view of the small number of seeds involved. The apparent reduction in the percentage germination resulting from both the yeast extract (Y) and hydrolysed casein (C) may be explicable on the basis of the toxic effects of amino-acids and amines (particularly L-tryptophane, nicotinic acid and nicotinamide) on seedling growth (Audus & Quastel, 1947).

The data presented in Table 1 show that the leaf mould (M) treatment caused a significant stimulation of growth, assessed on either a fresh- or dry-weight basis. This effect did not become apparent until the second seedling harvest, but from then onwards was consistently maintained. There was, in general, no significant difference in growth rate between plants of the I, C and Y treatments. The increased growth resulting from the M treatment is reflected in the mean weights of the whole plants, but the data obtained at harvests nos. 3, 4 and 5 show that it is the shoot and particularly the leaf growth that is enhanced. At harvest 4 the increase in leaf growth for the M treatment is highly significant. There is no significant difference in stem weight: a result related to the delayed onset of 'bolting' in the M plants. The fresh weight of the roots of the M plants significantly exceeds that for the I and Y treatments, but only equals that for the C treatment and on a dry-weight basis the C treatment roots are significantly heavier than those of the M, I and Y treatments. At harvest 5 the increase in leaf weight resulting from the M treatment is again highly significant. Now, however, the weight of the inflorescence and stem is also significantly greater with the M treatment, a result related to the significantly greater height of the seed stalk and the more rapid development of the inflorescence achieved by this date. The small increase in mean root weight recorded for the M treatment is not significant on either a fresh- or dry-weight basis. The stimulation of shoot growth by the M treatment is emphasized if the shoot/root ratios are calculated on either a fresh-weight (F) or a dry-weight (D) basis:

TABLE 3.

Harvest		Shoot/root ratio			
		M	I	Y	C
4	F	2·60	2·35	2·38	2·07
	D	2·58	2·08	2·07	1·75
5	F	3·70	2·96	2·86	2·72
	D	3·91	3·44	3·03	2·97

These data also show that there is a regular decrease of shoot/root ratio when the treatments are arranged in the order M–I–Y–C.

The data on moisture contents presented in Table 2 show that the differences between treatments were only significant for the stem and inflorescence at harvests 4 and 5 and for the roots at harvest 4, although the mean moisture contents for the M treatment plants were consistently higher than those for the other treatments (with the single exception of the leaves at harvest 4 when the values for M and I were almost identical).

The increased yield from the plants receiving the M treatment indicates that the leaf-mould extract supplied, either directly or indirectly, some substance or substances beneficial to growth. The existence of an indirect effect can only be established by a study of the sand micro-flora and by carrying out sterile cultures. The leaf mould may have exerted a direct effect either by supplying nutrients or by influencing the absorption of ions from the inorganic culture solution.

The lack of response to the yeast extract or hydrolysed casein may indicate that neither vitamins of the B group, nucleic acid derivatives, nor amino-acids are responsible for the beneficial action of the leaf mould. It should, however, be borne in mind in this connexion that other factors supplied by the Y or C treatments may have nullified any stimulating effect of these substances.

The correlation of the enhanced growth resulting from the M treatment with a higher shoot/root ratio and with a tendency to increased succulence might suggest a difference in nitrogen nutrition (Woodman, 1939*b*). Differences in inorganic nutrition will be revealed by the analysis now being undertaken on the dry-plant material. The present results, however, do not indicate any gross difference in absorption of macro-inorganic nutrients. The supplements M, Y and C were standardized on their nitrogen contents ($\equiv 6{\cdot}65$ p.p.m. N in treatment solution) and all plants received an adequate supply of inorganic nitrogen ($\equiv 32{\cdot}97$ p.p.m. N). Woodman (1939*b*) only recorded consistent increases in top/root ratio as the inorganic nitrogen content increased from 0·41 to 16·48 p.p.m., and there was no significant change in the ratio with 32·97 as compared with 16·48 p.p.m. Čajlachjan (1945) classifies lettuce as nitropositive or responding to supplementary nitrogen by earlier flowering, an effect not marked in the present investigation. It therefore seems unlikely that the results obtained are related to any quantitative difference in nitrogen uptake.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Monday, 22 March 1948, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, in the Chair.

*Symposium on biological aspects of water pollution**

The following papers were read and discussed:

Introduction. By Mr B. A. Southgate.

Some microbiological problems in the treatment of sewage and trade waste. By Dr L. A. Allen.

The biology of a percolating filter. By Mr T. G. Tomlinson.

Insect destruction of the filter film and a compensating factor. By Dr Ll. Lloyd.

The effect of pollution on fisheries. By Mr F. K. T. Pentelow.

Ordinary Meeting of the Association held on Friday, 23 April 1948, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, in the Chair.

Symposium on wool research

The following papers were read and discussed:

The organization of wool research. By Mr R. J. Bray.

The organization of wool education. By Mr P. A. Wells.

Some problems of wool growth. By Dr A. B. Wildman.

The protection of wool against attack by insect pests. By Mr J. Barritt and Mr R. S. Hartley.

THE ORGANIZATION OF WOOL RESEARCH

By R. J. Bray, *International Wool Secretariat, Dorland House, London, S.W.* 1

Wool is one of the oldest of the textile fibres, and owing to the continuous invention of new types of synthetic fibres, having some of the desirable properties of wool, its position as the most important textile fibre is no longer as secure as it was once thought to be. As a result, research is directed towards improvement in the quality of wool and in reducing costs of production. This work was commenced in various centres throughout the world in the 1920's.

The main wool-producing countries in the world and their approximate annual productions in millions of pounds are: Australia 1000, Argentine 510, U.S.A. 360, New Zealand 360, South Africa 210.

In Australia and South Africa, and to a somewhat less extent in the Argentine, the predominant breed of sheep is the merino, which produces the finest type of wool used in commerce. New Zealand and the United States produce what are known as the cross-bred types of wool which are coarser and longer than the merino types and less valuable.

* See *Nature, Lond.*, **161**, 874 (1948).

Main problems in wool production

The main problems in wool production which are at present the subject of research are: (i) development of increased uniformity of fibre in the fleece which is, of course, influenced by genetic and environmental factors; (ii) development of increased yield of fleece per sheep which again is influenced by genetic and environmental factors; and (iii) the study and control of parasitic diseases of sheep and diseases produced by nutritional deficiency.

(1) *Improving fibre uniformity*

The important fibre characteristics in wool manufacture are fineness, length and crimp or waviness. The variation of fibre fineness and the length in the fleece is roughly of the Gaussian type with a cephalo-caudal gradient and often also, a dorsi-ventral gradient. In other words, wool tends to get coarser and longer as one moves from the shoulder region to the britch of the sheep. Research is going on at various centres designed to improve fleece uniformity, both by reducing the fibre-to-fibre variation in fineness and length and also by reducing the cephalo-caudal and dorsi-ventral gradients in these characteristics of the fibre. The effect of inheritance and environment on these fleece characteristics is being studied.

(2) *Increased yield per sheep*

The most important factors influencing fleece weight are: (*a*) fibre length and diameter; (*b*) fleece density, that is, the number of fibres per unit area of the skin; (*c*) the total area of skin of the sheep.

(*a*) Increasing *fibre length and diameter* might appear to be an obvious method of increasing fleece weight. However, increased fibre diameter and length both lower the quality of the wool from the spinning point of view and hence reduce the price per lb. There is, often, too, an inverse correlation between the fibre diameter or length and fibre density. That is, an increase in fibre diameter or fibre length does not always mean an increase in fleece weight.

(*b*) The subject of *fleece density* itself is being studied by investigation of follicle arrangement in the skin and also by studies of fibre numbers and types in the growing fleece of breeding animals.

(*c*) *Increasing the skin area* as a means of increasing fleece weight has received most attention in the merino sheep and has been achieved by developing a wrinkled and folded skin which hangs in folds round the neck and shoulders of the more developed type of animal. There are, however, several drawbacks in the over-developed type of fleece of which the most important are: variation of fibre quality within the folds as compared with on top of the folds; increased susceptibility to blowfly strike; and difficulties in shearing. The latest tendency is towards a more plain-bodied sheep with a blocky rectangular body conformation so as to give a large skin area, and this has been coupled with an increased fibre density.

The subject of *fleece weight variation*, without reference to its detailed structure, has been tackled by what one might term 'operational research methods'. Thus, McMahon in New Zealand has analysed, by statistical methods, variation of fleece weights of pure bred flocks of Romney sheep maintained under various types of farming conditions. He has made quantitative estimates of the amount of variation due to heredity, environment, age of sheep, etc., from which it is possible to predict the increase in fleece weight likely to be obtained by improved breeding programmes, improved nutrition, etc.

Mention must also be made of the technique of *progeny testing* whereby a potential stud ram is tested for his ability to pass on his good characteristics to his progeny before he is used for commercial stud purposes. That is, of course, a logical development of the fact that many fleece characteristics are determined by Mendelian inheritance to a considerable extent. The technique is, however, difficult and complex with sheep but is being actively studied in the main sheep-rearing countries.

(3) *Parasitic diseases and diseases due to nutritional deficiency*

Of the *parasitic diseases*, probably the most important are blowfly strike, intestinal worms and foot rot, in that order. Among the other diseases of less importance there are a number of external parasites such as ticks, keds and mites which are controlled by dipping sheep in the appropriate insecticidal solutions.

Of the *nutritional diseases*, drought is probably the worst and its control, of course, is largely a question of economics of irrigation and of supplementary feeding. Of the trace element diseases, those due to cobalt and copper deficiency are the worst. Wool is a sensitive indicator of the sheep's condition, and the above deficiencies are reflected almost immediately by a thinning down of the fibre or even a complete break across the locks of wool. Such breaks are most undesirable from the manufacturing point of view and seriously impair the quality and value of the fibre. Cobalt deficiency has been a serious problem in several areas of the world. Coast disease in Australia; swayback disease in Derbyshire; pining sickness in Scotland, are diseases which have proved to be due to cobalt deficiency. These discoveries have been made during the past 20 years. Copper deficiency is also a serious problem in parts of Australia and has profound effects on the wool. Crimp or waviness of the fibre disappears and the wool acquires a straight, lustrous appearance and has been termed 'steely' wool. Both copper and cobalt deficiencies can be controlled by the administration of inorganic salts of the appropriate metal to the sheep in the form of salt licks, top dressing of pastures or by the addition of these salts in the supplementary feeding ration. The reaction to these treatments is extremely quick. For instance, the administration of copper salts in the supplementary feeding ration of sheep possessing 'steely' wool will cause the wool to resume its normal crimp and character within a few days.

The main wool research centres

The countries primarily interested in wool research are Australia, South Africa, New Zealand, the United Kingdom and the United States.

Australia is the country in which wool research is most elaborately organized. This is not surprising in view of the fact that wool production is Australia's largest industry. Research is being carried out to a greater or less extent on all the problems which have so far been considered. A reserve fund of several million pounds which accumulated from the wool industry during the war has now been set aside for the capital equipment of existing wool research institutes and for the setting up of new institutes. In addition, the Government now provides an annual grant of about £300,000 per annum for wool research, of which about two-thirds is to be spent on the biological problems involved in wool production. Also, a levy of two shillings is made by the Government on every bale of wool produced, and the resulting fund of about £300,000 p.a. is used partly to finance wool production in Australia, and also to provide a large part of the finance required for the activities of the International Wool Secretariat which allocates funds for wool research work and scholarships at wool centres all over the world. The organization of wool research in Australia is mainly in the hands of the Council for Scientific and Industrial Research, through its Wool Division. The C.S.I.R. is now placed in the invidious position of having vast sums of money available for wool research but being able to obtain neither equipment nor sufficiently qualified staff in the quantity and numbers required to embark on the greatly enlarged programme it has in view. It is, however, making an effort to remedy this situation by sending research officers and young University graduates for specialist training overseas. But men with the requisite knowledge and experience of wool research problems are few and far between, and it will take many years before these research projects can be fully organized. There is, however, an assured future for research workers, in Australia, qualified to take up the problems of wool research, for much of the training can, of course, be arranged in the United Kingdom in many of the requisite subjects.

In *South Africa* the predominant breed of sheep is the merino and the problems requiring

investigation are, therefore, much the same as in Australia. Research is, however, on a much smaller scale and is mainly concentrated in the South African Department of Veterinary Science and Animal Industry at Onderstepoort which has a separate Wool Section. The Grootfontein College of Agriculture also does wool research. Both of these Departments undertake investigations in improving the fleece characteristics of the merino, body conformation, fleece density, effect of nutrition on wool production, etc.

In *New Zealand* the predominant breed of sheep is the Romney, which is a bigger sheep than the merino and has a longer, coarser fleece, although the average fleece weight is much the same in both breeds, that is, about 10 to 12 lb. Research has, therefore, mainly been carried out with the Romney breed. There are two main research centres, Massey College in the North Island and Lincoln College in the South Island. At Massey College attention is mainly directed towards genetical problems, particularly the inheritance of fleece characteristics in Romney sheep. Mendelian inheritance of certain types of hairy birth coats of Romney lambs has been demonstrated in this breed of sheep at Massey College. At Lincoln College, statistical methods have been used to estimate variation in fleece weight of flocks of Romney sheep due to genetic factors, environmental factors, age of sheep, etc., as indicated previously.

In the *United Kingdom* biological research on wool is undertaken by the Biology Department of the Wool Industries Research Association. Here, investigations undertaken include: development and microscopic structure of the wool fibre; studies of fleece growth in the young lamb and its relation to changes in the follicle population of the skin. An experimental Romney flock is maintained by W.I.R.A. at the Institute of Animal Nutrition, Cambridge University, where intensive investigations are being made of the effect of age, seasonal changes in nutrition and the reproduction cycle on such fleece characters as fibre length and diameter, fibre density, fleece weight per unit area of the skin, etc. The inheritance of these characters is also being studied. The Department also co-operates with the North of Scotland College of Agriculture in investigating problems of wool production in Scottish Blackface sheep. Some research is done by other organizations in the United Kingdom, particularly on parasitic and deficiency diseases of sheep. This work usually forms part of the general veterinary programme of the various agricultural institutes.

In the *United States* very little organized research is done on wool. The U.S. Department of Agriculture has a wool laboratory, but it is mainly concerned with research into methods of measuring fibre diameter and length in wool fibres and their relation to various commercial grades of wool. Certain of the agriculture experiment stations include some investigations on wool in their general programme of animal management, but on the whole, research has been directed more to the general problems of sheep management than to those concerned specifically with wool. A Wool Advisory Committee of the United States Department of Agriculture has recently been formed, and in its first report it has recommended that a research programme of wool improvement should be carried out, particularly with reference to the effect of sheep nutrition on wool production. So far, very little organized research of this type has been carried out in the U.S.A.

THE ORGANIZATION OF WOOL EDUCATION

By P. A. Wells, *International Wool Secretariat, Dorland House, London, S.W.* 1

The wool research worker has been drawn mainly from the ranks of those who have graduated in pure chemistry, physics or biology. Many technologists, directors, managers, designers, merchants and others, including foremen and operatives, have, however, followed courses of applied knowledge relating to wool, especially at the technical colleges and schools. It is the object of this brief survey to outline both the content of wool education, exclusive of research, and the educational system providing it. In the space available it will be possible to consider only the United Kingdom.

The content of wool education

The content of wool education may be considered under the following heads:

Wool growing, including sheep breeding and raising.

Supply and marketing of wool, including the commercial and economic factors involved.

Design and manufacture, including the manipulation and processing by hand or machinery, of fabrics and yarns from raw wool, and the selection of appropriate types and qualities.

Science of wool, from the standpoints of biology, chemistry and physics.

Each of the above groups may be greatly expanded. Thus manufacture may be subdivided into the manipulation of wool fibres into (1) woollen, or worsted, woven fabrics—the yarns of the latter, unlike the former, composed of more or less parallel fibres so as to present a clear-cut rather than a 'fuzzy' appearance; (2) knitted fabrics which, instead of the warp and weft of woven cloths, are each made up of a single, continuous yarn; (3) pile fabrics, such as carpets, in which the cut or looped ends of the yarns all project on the same side; and (4) felts, in which the fibres are intermingled and compressed into a sheet.

The production of these various types of fabrics requires a number of widely differing processes. Thus, to make woollens, amongst other processes, the raw wool is sorted into various qualities; scoured or washed to remove grease and dirt; two or more qualities may be blended together; 'carded' to open out the wool into a thin gauze-like veil; spun into yarn; and the yarns woven or knitted. For worsted yarns and cloths the carded wool is combed and drawn out, before spinning, to remove the short fibres and render the remainder parallel. To make felt the carded wool is subjected to pressure and steamed until it forms a sheet of densely entangled fibres.

The scoured wool, yarn, or fabric may be dyed at some suitable stage of processing and, finally, the cloth is given various finishing treatments to achieve particular effects or, for example, to moth-proof it. Such finishing processes also include 'raising' or brushing up the surface fibres to produce a nap, or 'cropping' the surface fibres to produce a clear effect.

The wool educational system

The wool educational system in this country has in the main, like the British constitution, evolved and has only to a small extent been planned. Thus apprenticeship dates back to eleventh- and twelfth-century Guilds such as the Woollen Cloth Weavers. The various organizations which compose its structure may be broadly classified according to their functions and educational levels, and are generally located in the corresponding wool manufacturing districts.

Universities. The Department of Textile Industries at Leeds University provides courses on Worsted and Woollen Yarns, Designing, Handloom and Powerloom Weaving, and Finishing; while the Department of Colour Chemistry and Dyeing covers the dyeing of loose wool, yarns and fabrics. Instruction is also given in Textile Testing, and Textile Chemistry and Physics, including the X-ray examination of the structure of wool fibres. These studies lead to the Ordinary and Honours Degrees of B.Sc. in Textiles, or to the Diploma in Textile Industries. In addition, there is a course for the Degree of B.Com. with Textiles as principal subject, which covers the economic and commercial aspects of wool.

Technical colleges. Education in wool manufacture, technology and science is provided by a number of technical colleges, many of which, however, are biassed towards the particular branch of the wool industry preponderating in the area concerned.

Thus courses on woollens are available at the Technical Colleges of Batley, Belfast, Dewsbury, Morley and Stroud; and the worsted side is specially catered for at Bradford and Halifax. Teaching of both woollen and worsted manufacture is done at Glasgow Royal Technical College, the L.C.C. College for the Distributive Trades, Huddersfield and Keighley, while the Scottish Woollen Technical College at Galashiels specializes in tweeds. The Galashiels institution offers courses by correspondence as well as by attendance, and also has affiliated evening classes at Aberdeen, Alloa, Alva, Elgin, Inverleithen, Langholm and Peebles.

There is a proposal to set up a National Wool College* at Bradford to provide a centre for high-level teaching including both woollens and worsteds. Objections have been raised, however, by the woollen districts on the score that Bradford is a predominantly worsted area.

Knitted fabrics, such as hosiery, are covered by courses at Leicester College of Technology, Loughborough College and Nottingham and District Technical College. Carpet weaving is taught at Halifax Technical College.

Among some interesting special courses are 'Fancy Woollens, Rugs and Tapestries' at Belfast, 'Design of Men's Wear, Dress, Furnishing and Decorative Fabrics' at Keighley, and 'Mildewing and its Prevention', at Loughborough. In addition, Glasgow has a course in 'Cloth Costing and Distribution', and Bradford has a course for merchants and others on the distributive side of the wool textile industry.

Amongst the qualifications relating to wool for which students are prepared by the institutions which have been mentioned are the Associateship (A.T.I.) and the Fellowship (F.T.I.) of the Textile Institute; the examinations of the City and Guilds of London Institute, leading to Associateship of the Institute (A.C.G.I.); and of the East Midland Educational Union; the National Certificates in Textiles; and the Certificate of the Hosiery Manufacturers' Association. In addition, College Diplomas are awarded by Bradford, Huddersfield, Galashiels and Glasgow, the last-named also awarding an Associateship of the College.

Technical and art schools. The technical and art schools operate on similar lines to the colleges, although they are smaller and rarely undertake research. Thus woollens are dealt with at Rochdale Technical School, while instruction on both woollens and worsteds is given at the technical schools of Saltaire and Selkirk, and at the Victoria Technical Institute, Trowbridge. The Henderson School of Technology, Science and Art at Hawick has courses on hosiery; while the Kidderminster and District School of Science and Art is developing a course on wool dyeing and finishing for the carpet industry.

A number of these schools, such as the Scarborough Technical Institute and the Regional College of Art at Bradford provide courses relating to wool in the form of hand spinning, hand weaving, dyeing with vegetable dyes, or textile design and colour. Two interesting private institutions may also be mentioned here: the London School of Weaving and Kensington Weavers (Associated) which provides practical instruction in hand carding, spinning, weaving, cloth design and vegetable dyeing; and the Tailor and Cutter Academy, which gives practical training in the cutting and making-up of woollen and worsted cloths into suits and dresses.

In addition, education in textiles, including wool textiles, is given at a number of secondary technical schools.

Scientific, technical and educational organizations. Certain scientific societies provide education in wool by means of lectures and publications, and of these the Textile Institute admits Fellows and Associates after due training and examination, and a Joint Committee with the Ministry of Education administers the award of Ordinary and Higher National Certificates in Textiles. A proposal has recently been made to set up a Clothing Institute* on similar lines to the Textile Institute. Patons and Baldwin's Textile Institute at Alloa prepares students for the City and Guilds of London Institute's examinations in wool manufacture. Various local textile societies, such as the Bradford Textile Society, provide lectures biased towards wool if located in wool districts, and are connected by affiliation to the Federation of Textile Societies and Kindred Organizations.

The Society of Dyers and Colourists, through its Education Committee, publications and lectures; the Guild of Calico Printers', Bleachers', Dyers' and Finishers' Foremen, and the Guild of Technical Dyers, foster education relating, *inter alia*, to wool and wool dyeing.

The institutions previously described are mainly interested in the education of students and apprentices or the further education of technologists. The education of children, as well as laymen, is the chief concern of the Education Department of the International Wool

* Now instituted.

Secretariat. The Department of Education has a panel of trained, graduate teachers who tour the country giving lectures, in co-operation with local education authorities, on such topics as Wool Growing, Marketing, Manufacturing and the Science of Wool, illustrated by films and filmstrips. Other visual aids include a chart showing manufacturing processes from the sheep's back to the finished fabric; a display card bearing actual samples of wool at different stages of processing; and a map of the world showing sheep distribution and wool production and consumption. In addition, books are being published for use in primary, secondary and further education—the three groups into which the British educational system may be divided. During the past twelve months over 64,000 persons heard the story of wool while, by means of lectures in the Teachers' Training Colleges, it is hoped to provide wool education, indirectly, for children and adults throughout the country. In addition, school lectures on hosiery are conducted by the Leicester and District Hosiery Manufacturers' Association Ltd.

A somewhat similar function is performed by the Wool (and Allied) Textile Employers' Council, but with a strong bias towards training and labour recruitment publicity for the wool textile industry in the West Riding.

Examining bodies. Mention has been made of certain examining bodies. The City and Guilds of London Institute, however, is an examining body only. It includes such subjects as Woollen and Worsted Manufactures, Manufacture of Hosiery and Knitted Goods, and the Dyeing of Wool, and holders of the Full Technological Certificate may be admitted to Associateship.

Advisory bodies. There are a number of mainly advisory, investigating or co-ordinating bodies, for example, the National Council of Technology,* which is in process of formation; and Regional Advisory Councils for Technical Education, such as the Yorkshire Council for Further Education, some of which are closely concerned with wool and are likely to play an increasingly important part in the future.

Scholarships. Scholarships for education and training in wool are awarded by such bodies as the Textile Institute, the Textile Machinery Makers Ltd., the International Wool Secretariat, and the Chambers of Commerce of various towns located in areas devoted to the wool industry.

There are no Degree or Diploma courses in Wool Textile or Textile Engineering,† or in Textile Administration, although these subjects exist in one form or another as ancillary studies. There are, too, no independent courses of instruction in Feltmaking, although it forms part of a course on Textile Industries at Leeds University; and a course in felt hat-making has been initiated at Stockport College for Further Education. The foregoing survey indicates, however, the otherwise considerable facilities available in the United Kingdom for education in wool from sheep to suits.

SOME PROBLEMS OF WOOL GROWTH

By A. B. Wildman, *Wool Industries Research Association, Torridon, Headingley, Leeds*

The main objectives of this branch of wool science are: first, to increase our knowledge of the structure of wool. It is necessary not only to be able to distinguish wool from other fibres, but to learn what parts of the fibre contribute and in what way they contribute to the special behaviour of wool during its conversion into fabrics.

Secondly, the requirements of the wool manufacturer must be defined in terms of measurable fibre characteristics. The way in which these characteristics vary and what causes the variation must be followed up so that we may be able to influence production towards the greatest amount of uniform wool of the desired type. There is no single best type of wool: fine merino wool, for example, is suitable for the manufacture of fine worsted suitings but is quite unsuitable for carpet-making.

* Now formed.

† A Chair of Wool Textile Engineering Research has now been instituted at Leeds University.

Thirdly, it is necessary to know how fibres are produced in the skin, so that we may understand by what means the variations in fibre and fleece form are brought about. Research in wool biology may be divided broadly, therefore, into the following categories:

I. Fleece investigations.

II. Studies on follicle arrangement and populations.

III. Skin activity and the growth and anatomy of fibres and follicles in the skin.

IV. The microscopy or morphology of mature fibres.

The principles which have emerged from a fleece investigation carried out by The Wool Industries Research Association, using Romney sheep, were then outlined. The connexion between rate of skin expansion and mean fibre length and numbers of fibres growing per unit area of skin (fleece density) was explained. Differences found in different sheep and body regions in the variation in thickness and length from fibre to fibre were illustrated. This is a genetic variation, that is, it is a property of the individual sheep or region. There is also a variation in thickness along the lengths of each fibre and this is caused by environmental influences.

The potentialities of different sheep for wool production, however, cannot be fully understood from the results of fleece investigations alone: if a study of follicle populations is made, the proportion of the total number of follicles which are actually producing fibres can be determined, as well as those follicles which are dormant and those which are shedding fibres.

A further type of investigation, to which reference has already been made, is the detailed study of cell changes within the follicle and the growing fibre. The relative speeds of cell differentiation and keratinization must influence the final shape of the fibre and this is an important factor contributing to the variation in fleece form. In some developing fibres, keratinization proceeds eccentrically and this condition is associated with an eccentric position of the fibre within the follicle. Recent work at Torridon has revealed how the cuticular scales and the fibrillae of the cortical cells of some wool fibres are formed. For a proper understanding of the principles of wool growth, it is essential that these different branches of wool biology should be linked and studied concurrently.

THE PROTECTION OF WOOL AGAINST ATTACK BY INSECT PESTS

By J. Barritt and R. S. Hartley, *Wool Industries Research Association, Torridon, Headingley, Leeds*

An increasing interest has been shown in recent years by the general public, manufacturers and the Government, in the damage caused to wool and related fibres by clothes moths and other keratin-eating insects. This is due in part to the present shortage of clothing and also to an increased awareness that methods exist of combating those pests which damage wool.

No reliable estimate of the losses due to clothes moths and carpet beetles can be given but it must, throughout the world, run into many millions of pounds per annum; hence the importance of finding methods of preventing such damage (Hartley, Elsworth & Barritt 1943).

Any insect which can bite through a wool fibre can cause damage, especially to a knitted fabric. A distinction must be drawn, however, between those which cause accidental damage, such as crickets and cockroaches, and those which can feed on keratinaceous fibres. In the latter group are some seven or more species of moths and some seven or more species of Dermestid beetles which can utilize keratin as food material (see Appendix).

In this country the clothes moths, in particular *Tineola bisselliella*, are more important than the Dermestid beetles. In North America the carpet beetles *Attagenus piceus* and *Anthrenus vorax* are regarded as of equal importance to clothes moths, in the near East and India the 'Woolly bear', the larva of *A. fasciatus*, is an important pest, whilst in the Antipodes, *Anthrenocerus australis* appears to be the dominant pest.

All the true keratin-eating insects have several characteristics in common: (1) the larva is responsible for the damage and not the adult; (2) the larva can feed on dry animal protein material (wool in equilibrium with the atmosphere contains from 10–15% of water) and obtain its necessary water from the breakdown, during digestion, of the food material.

Linderstrøm-Lang & Duspiva (1935) have suggested the existence of the following mechanism for the breakdown of keratin by *Tineola bisselliella*: The intestinal fluid contains an agent capable of reducing the disulphide cross-linkage of the wool fibre, thus rendering the fibre more susceptible to breakdown by enzymes, e.g. proteinases, present in the intestinal fluid. The work of Geiger, Kobayashi and Harris (1942), who found that replacement of the disulphide linkages by bis-thioether linkages increased the resistance of wool to attack by moth and carpet beetle larvae may be regarded as supporting this hypothesis. A method of mothproofing wool, based on the findings of Geiger *et al.* (1942), has recently been patented (U.S. Patent 2418071).

The carpet beetles appear to attack a wider range of materials than the clothes moths; the latter can, however, be reared on diets free from keratin, such as fish meal, casein plus yeast, cholesterol and mineral matter, and wholemeal flour. Clean wool in the manufactured state is not an adequate diet for *T. bisselliella*. Soiling with perspiration, urine and food improves its dietetic properties. The absence of Vitamin B and possibly sterols may explain the inadequacy of clean wool.

General methods for protection

Three general methods of affording protection are available: (1) the periodic spraying of wool goods and storage places with a fluid insecticide or dust; (2) storage of the wool in an atmosphere inimical to moth life (this calls for a closed chamber or package and a volatile substance); (3) addition to the wool of some substance which renders it immune from attack.

Method 1. Insecticidal sprays, often described as solvent contact sprays, are used primarily for combating insect infestations in buildings and storage places. Those based on pyrethrum or related compounds alone give only temporary protection. If, on the other hand, D.D.T. is incorporated in the fluid and a sufficient amount is applied evenly to the goods, some measure of permanent protection can be obtained.

Alternatively, dusts based on D.D.T. or benzene hexachloride can be used; according to Burgess (1948) dusts are not so effective as sprays.

Method 2. The volatile substances that have been most generally used for creating an atmosphere inimical to moth life are paradichlorobenzene and naphthalene or mixtures of both. A recent development is the addition of Gammexane to the *p*-dichlorobenzene or naphthalene. These substances are only effective while the goods are stored in sealed containers or well packed in stout paper packages. It is obvious that the protection is only maintained so long as the vapour concentration itself is maintained.

Method 3. The most satisfactory method of mothproofing wool is to add to it some agent which renders the wool immune to attack by moth larvae. There are two lines of approach to this problem: (1) to add to the wool externally some substance which will render the wool indigestible or toxic to the larvae; (2) to modify the keratin structure internally so that it is no longer capable of digestion by the larvae. The latter method was used by Geiger *et al.* (1942).

Testing and breeding of larvae

When studying the behaviour of mothproofing agents it is essential to have a method of testing the efficiency of such agents. In the Wool Industries Research Association laboratories this is done by exposing the proofed material to attack by the larvae of *T. bisselliella*. In order that the test shall be reliable it is necessary to maintain stocks of larvae, succeeding generations of which can be relied on to behave in a similar manner. The method used is to grow the larvae on wool cloth impregnated with 10% of a mixture of ten parts brewers' yeast and one part cholesterol. The larvae are maintained in the dark and in an atmosphere

maintained at 70° F. (21·1° C.) and 65% relative humidity. The larvae are used for tests when they are 35–40 days old. The test unit consists of a brass tube 1¼ in. in diameter and 1 in. high, one end being closed with copper gauze. This is placed on a 2¼ in. square of the fabric to be tested, ten larvae being used per test, the duration of the test being 30 days. To ensure that the larvae are active and capable of eating wool, a control test on untreated cloth is carried out at the same time. At the end of the test the untreated cloth is badly eaten and a large amount of excrement is present. In a satisfactorily proofed material the damage and excrement are small. The condition of the larvae is important as a clue to the physiological action of the proofing agent.

Mothproofing agents used

The ideal mothproofer is one which can be applied easily during processing, has no deleterious action on the fabric and remains effective throughout the life of the material. It should in addition be cheap.

Mothproofing was first studied in Germany by Meckbach (1921) and other chemists employed by the Bayer Co. Possibly the first mothproofing agent to be recognized was 2:4-dinitro-α-naphthol (Martius yellow or Manchester yellow, S.D.C. Colour Index no. 9). The yellow colour of this material is a disadvantage for clothing and furnishings, as is also its poor washing fastness. Where colour and fastness are of no importance, e.g. in packaging felts, laggings, piano-key felts, dinitro-α-naphthol is very useful. It is non-corrosive to metals in contact with the wool and the colour can be used as a rough index of the amount of proofing agent present, and indicates the degree of penetration of thick hard felts. Dinitro-α-naphthol can be applied in the dye-bath or by impregnation of the fabric with a solution of requisite strength (i.e. padding). The minimum amount required to give satisfactory protection is of the order of 0·5%.

An important class of mothproofing compounds is based on the use of inorganic fluorides, for example, sodium fluoride, sodium silicofluoride, potassium bifluoride, chromium fluoride, potassium antimony fluoride, aluminium ammonium fluoride and many others. Broadly speaking, these compounds have poor washing fastness, otherwise they are excellent mothproofers. It has been shown by Hartley *et al.* (1943) that 0·2–0·3% fluorine is required for adequate protection. These compounds can be added to wool by impregnation, by exhaustion, and during dyeing. Proofing agents based on inorganic fluorides have been marketed under a number of branded names, i.e. Eulan M, Eulan Extra, Eulan W Extra, Kydo, Foudrex and others. A number of methods have been described for increasing the washing fastness of fluorine-based mothproofers.

Another substance which is being used for mothproofing is pentachlorophenol or its fatty esters, marketed as various brands of Mystox. Application to wool can be made by impregnation (i.e. padding) by exhaustion, during dyeing, and in dry cleaning. The amount of pentachlorophenol necessary to give protection to wool is 0·5% (Hartley *et al.* 1943). Like the fluorides this proofer has poor washing fastness.

All the mothproofing agents so far described have, to a greater or less extent, the disadvantage of poor washing fastness. The search for more durable mothproofers is essentially a record of the production from 1928 onwards of various Eulans (by the Bayer Co. and later I.G.) and in 1939 of Mitin F.F. (by the Geigy Co.). Eulan N, Eulan C.N. and C.N. Extra are derivatives of triphenylmethane, e.g. Eulan C.N. is understood to be the sodium salt of 3:3′–5:5′–4″–pentachloro–2:2′–dihydroxy triphenylmethane–2″–sulphonic acid, and Eulan N the sodium salt of 3:3′–5:5′–tetrachloro–2:2′–dihydroxy–triphenylmethane–2″–sulphonic acid. Mitin F.F. is a substituted urea, the effective grouping being *p*–*p*′–dichlorodiphenylether, a sulphonic acid group being present to confer solubility. These substances are colourless, and behave towards wool like acid dyestuffs (i.e. they are applied in an acid dye-bath). Another type of compound, Eulan N.K., which is triphenyl dichlorobenzyl phosphonium chloride, differs from the other Eulans in that it is applied in a neutral dye-bath.

Extensive and repeated washing tests under commercial conditions on blankets proofed with a number of mothproofers have shown that Eulan C.N. and Mitin F.F., although much more resistant to washing than other proofing agents, are gradually removed, the protection they offer not being unlimited. On certain kinds of goods which have long life and receive a limited amount of washing, such as suitings and furnishings, Eulan C.N. and Mitin F.F. will probably give protection throughout the life of the goods. On goods which are washed frequently the protection they give will be limited.

The most important recent development in mothproofing is the advent of D.D.T. This is the most potent substance so far investigated, as little as 0·1 % on the wool being sufficient to give adequate protection. On fat-free wool D.D.T. is slowly volatile; thus in practice 0·25 % is recommended. D.D.T. can be applied by impregnation, by exhaustion, to a limited extent during dyeing, and in dry cleaning. D.D.T. has poor washing fastness; if, however, 1 % is applied to the fabric, the fabric will withstand a number of washes before the D.D.T. content is reduced below 0·1 %.

Attention has been confined to those mothproofing agents which are being or have been used commercially, but it should be added that a large number of mothproofing agents have been described in the literature and new ones, or old ones refurbished, are constantly appearing.

Much work has now been done on mothproofing agents and mothproofing of wool is carried out on a considerable scale. Whilst it cannot be said that any one of the mothproofing agents described above fulfils all requirements, it can be said that apart from goods which are washed frequently, adequate protection from moths can be achieved.

APPENDIX

Lepidoptera

Tineola bisselliella (Humm.)	Common or webbing clothes moth
T. pellionella (L.)	Case-bearing clothes moth
T. flavescentella Haw.	——
T. lapella Hubn.	——
T. pallescentella Staint.	Large pale clothes moth
Trichophaga tapetzella (L.)	Tapestry moth
Hoffmannophila pseudospretella (Staint.) *Borkhausenia pseudospretella* (Staint.)	Brown house moth or false clothes moth

Carpet beetles

Attagenus piceus (Oliv.)	Black carpet beetle	Cosmopolitan: first recorded in Britain 1868
A. pellio (L.)	Fur beetle	Cosmopolitan
Anthrenus vorax Wath.	Furniture carpet beetle (U.S.A.)	N. America
A. fasciatus Herbst.	Woolly bear (India)	Oriental regions
A. verbasci (L.)	Varied carpet beetle	Cosmopolitan
A. scrophulariae (L.)	Common carpet beetle	Cosmopolitan
Anthrenocerus australis (Hope)	Australian carpet beetle	Australia, New Zealand; in Britain since 1933.

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